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## Synopsis of Freshwater Crayfish Diseases and Commensal Organisms

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### Abstract

Disease agents and pests associated with freshwater crayfish fall into six main categories—viruses, bacteria, rickettsia-like organisms (RLOs), fungi, protists, and metazoans. Data and information on specific disease agents and pests from each of these categories are presented in this synopsis. Each agent or group of agents is considered under the following headings—condition, causative agent(s), life cycle/life history, epizootiology, pathology, pathogen viability. Information for the synopsis was obtained from the published literature and from personal contact with internationally recognized experts in freshwater crayfish aquaculture, biology, and disease. Data of relevance for import risk analysis are summarized.

Import risk analysis is the process by which the risks associated with importation of animals and plants, and products derived from animals and plants, are assessed and managed. Hazard identification is essential and is the first component of an import risk analysis. In 1996, the Australian Quarantine and Inspection Service (AQIS) commenced a review of policy relating to the importation of nonviable freshwater crayfish products, along with a suite of other aquatic animal products. AQIS

commissioned a synopsis of freshwater crayfish pests and pathogens for use as a resource document for hazard identification in the formal IRA process.

**Keywords:** freshwater crayfish, diseases, pathology, import risk analysis

## 1. Introduction

Research studies on pathogens and commensals of freshwater crayfish have a long history. The introduction of the fungus *Aphanomyces astaci*, causative agent of crayfish plague, into Europe in the mid-1800s (Cornalia, 1860) led to an early research focus on crayfish diseases. Research interest in the pathogenesis and ecological consequences of crayfish plague continues to the present day (Cerenius and Söderhäll, 1992; Taugbol and Skurdal, 1993; Alderman, 1996). Similarly, *Psorospermium haeckeli*, recently classified as a protist (Ragan et al., 1996), was first described in 1857 (Haeckel, 1857) and still remains a topic of significant research endeavor (Thörnqvist and Söderhäll, 1993; Gydemo, 1996; Henttonen, 1996). Crayfish viruses, on the other hand, are a recent discovery, the first reports of a crayfish virus appearing in the early 1990s (Anderson, 1990; Anderson and Prior, 1992).

There have been numerous major (Unestam, 1973; Alderman and Polglase, 1988; Thune, 1994; Vogt, 1999) and minor (Johnson, 1977; O'Keefe and Reynolds, 1983; Mills, 1983, 1986; Vey, 1986; Smith and Söderhäll, 1986; Paynter, 1989; Owens and Evans, 1989; Anderson, 1990; Evans et al., 1992; Cerenius and Söderhäll, 1992; Gydemo, 1992; Nylund and Westman, 1992) reviews on crayfish diseases or general reviews containing sections on crayfish diseases (Sparks, 1985; Bower et al., 1994). These publications attest to the ongoing research interest of crustacean biologists and pathologists in the general area of crayfish pathology and commensal organisms.

The term "disease" has been defined as a "demonstrable negative deviation from the normal state (health) of a living organism" (Kinne, 1980). In this definition, Kinne uses the phrase "negative deviation" in terms of a functional and/or structural impairment that is quantifiable in terms of a reduction in ecological potential (i.e., survival, growth, reproduction, energy metabolism, stress response, endurance). The causes of disease are wide and varied and include both biotic and abiotic factors. However, in the context of the present synopsis, only biotic causes of disease are considered.

A commensal organism is one that lives in close association with another organism, deriving some benefit from this arrangement but causing no harm to the host. Crayfish commensals are found on the exoskeleton, including the branchial chamber (Johnson, 1977; Alderman and Polglase, 1988). Organisms that reside in the gut of crustaceans, including freshwater crayfish, have also been described (Mickeniene, 1983; Harris, 1993; Evans et al., 1992). Whether some, or most, of the commensal organisms found on or in crayfish should be described as "commensals" or "symbionts" is a matter of debate. Many of the organisms described in the published literature as commensals have highly specific host-commensal associations. It is likely that such organisms are dependent on the host crayfish for specific nutrients or other life-process requirements. They are unlikely to be able to survive for extended periods away from the host. For these reasons, such organisms are probably better described as symbionts rather than commensals.

While some crayfish pathogens and symbionts have been studied in considerable detail, information on most disease agents, disease conditions, and symbiont associations is lacking. With the growth of crayfish aquaculture worldwide, the occurrence of disease outbreaks is likely to increase. This development will create pressure for investigations on the etiology, pathogenesis, prevention, and treatment of crayfish diseases. A stimulus for these types of studies also comes from an increased awareness of the environmental consequences of introduction of exotic organisms into aquatic ecosystems. Such pressures will enhance the research focus on crayfish pathogens, symbionts, and commensal organisms and provide valuable data and information for formulation and ongoing review of quarantine policy on importation of live crayfish and crayfish products into Australia and elsewhere.

Disease-causing organisms that are seen as posing a threat to crayfish or other aquatic groups fall into six main categories—viruses, bacteria, rickettsia-like organisms (RLOs), fungi, protists, and metazoans. These six groups are dealt with sequentially in this synopsis. For clarity and ease of reference, the information pertaining to each agent or group of agents is arranged under the following headings:

- Condition
- Causative agent(s)
- Life cycle/Life history (if relevant)
- Epizootiology
- Pathology
- Pathogen viability

## 2. Viruses

Viruses comprise a conspicuously understudied group of freshwater crayfish pathogens. In fact, the first natural viral infection of a freshwater crayfish was described only recently (Anderson and Prior, 1992). Since this discovery, four new freshwater crayfish viruses have been reported. There have also been descriptions of viruses important in other aquaculture industries that experimentally or naturally infect freshwater crayfish. The viruses found only in freshwater crayfish have not been studied beyond preliminary descriptions of their morphology, with notes on their morphogenesis, and rarely their nucleic acid composition. No freshwater crayfish virus has been purified to compare physicochemical characteristics with other viruses or to produce highly specific and sensitive molecular diagnostic tools.

### ***2.1. Condition: infectious pancreatic necrosis virus (IPNV) infection***

IPNV causes acute disease in salmonids. Consequently, IPN disease and virus viability has been thoroughly reviewed in preparing IRAs for salmonids (e.g., DPIE, 1997). Only information relevant to IPNV in freshwater crayfish will be presented.

#### *2.1.1. Causative agent*

IPNV is a birnavirus (Halder and Ahne, 1988).

### 2.1.2. Epizootiology

IPNV strain Sp was experimentally transmitted to the native European crayfish *Astacus astacus* by injection, waterborne and oral (in feed) exposure, and by cohabitation (shared water) with infected fry of rainbow trout, *Oncorhynchus mykiss* (Halder and Ahne, 1988). Furthermore, the experimentally infected *A. astacus* continually excreted IPNV, and fry and eggs of *O. mykiss* were infected by exposure to effluent water from tanks containing the inoculated crayfish. Halder and Ahne (1988) were not able to prove that IPNV replicated in *A. astacus*, and postulated that *A. astacus* was a mechanical vector for IPNV. IPNV was isolated from *A. astacus* haemolymph up to 1 year post-inoculation (injection, oral, and waterborne exposure).

### 2.1.3. Pathology

IPNV-infected *A. astacus* do not exhibit clinical disease. High titers of IPNV were isolated from all tissues of *A. astacus* inoculated by injection for which virus isolation was performed at death (Halder and Ahne, 1988). Those tissues tested included muscle, stomach, gills, hepatopancreas, antennal gland, gut, heart, and gonads. No histopathology or cytopathology was observed. However, IPNV was observed in haemocyte granules.

### 2.1.4. Pathogen viability

The viability of IPNV in crayfish tissues under varying physicochemical conditions has not been studied.

## 2.2. Condition: white spot syndrome virus (WSSV) infection

### 2.2.1. Causative agent

WSSV is approximately 350 × 100 nm (Richman et al., 1997) and is rod shaped to elliptical (Inouye et al., 1994; Wongteerasupaya et al., 1995; Inouye et al., 1996; Durand et al., 1997). Negative staining of purified WSSV reveals that the cylindrical nucleocapsid is striated at 90° to the longitudinal axis (Wang et al., 1995; Wongteerasupaya et al., 1995; Inouye et al., 1996; Durand et al., 1997) and the nucleocapsid has a tail contained in a longitudinal expansion of the envelope (Wang et al., 1995; Wongteerasupaya et al., 1995; Durand et al., 1997). The WSSV genome is a nonsegmented, dsDNA molecule greater than 150 kbp in length (Wang et al., 1995; Inouye et al., 1996). Recent research suggests that WSSV is either a member of a new genus in the Baculoviridae, tentatively named "Whispovirus," or a member of a new virus family (van Hulten et al., 2000a,b).

### 2.2.2. Epizootiology

WSSV complex has been associated with epizootic mortality in prawn aquaculture throughout Asia and has been introduced to North and South America (Lightner, 1996; Jory and Dixon, 1999). WSSV has been spread mostly by movement of live prawns, but the outbreak of WSSV in the USA may have been due to introduction of the virus from waste from a plant that was processing frozen prawns from Asia (Lightner et al., 1997).

WSSV infects many penaeid species (Chou et al., 1995; Wongteerasupaya et al., 1996) as well as many nonpenaeid crustaceans including freshwater crayfish and prawns, crabs,

and marine crayfish (Lo et al., 1996; Flegel, 1997). Recently, WSSV was associated with mortality up to 90% and up to 20–30% in *Orconectes punctimanus* and *Procambarus* sp. respectively originating from southeastern USA (Richman et al., 1997).

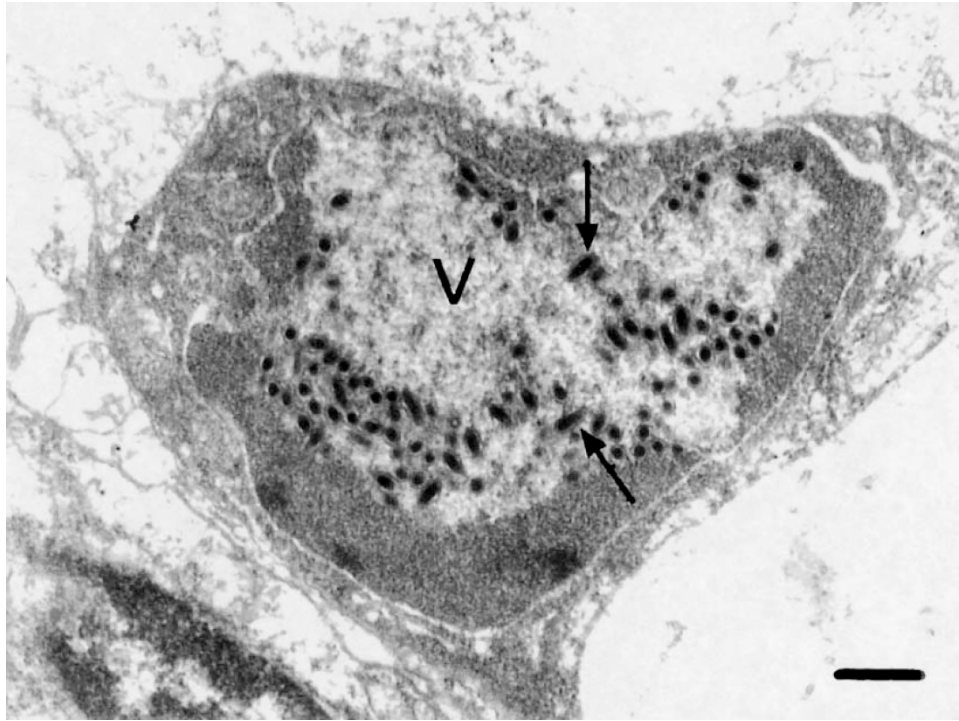
WSSV may cause 100% mortality in *Penaeus monodon* ponds 2–7 days after the onset of disease (Chou et al., 1995). WSSV is transmitted through water and per os by cannibalism (Chou et al., 1995; Wang et al., 1998). Morbidity usually occurs within 5–7 days post-inoculation. WSSV was transmitted to *Procambarus clarkii* by feeding with infected *P. monodon*, resulting in a cumulative mortality of 81% by 18 days after feeding (Wang et al., 1998).

### 2.2.3. Pathology

Clinical signs of WSSV infection of *Orconectes punctimanus* and *Procambarus* sp. included discoloration and mottling of the exoskeleton, primarily on the carapace and occasionally on the chelipeds (Richman et al., 1997). Characteristic small white spots develop on the inside surface of the cuticle of the carapace and appendages of infected prawns (Chou et al., 1995). Such prawns are also lethargic, and often red in color (Nakano et al., 1994; Chou et al., 1995). *Scylla serrata* display the same clinical white spots (Lo et al., 1996). However, clinical symptoms in other crustaceans are often absent.

WSSV infects haemocytes (Momoyama et al., 1995) and other tissues of mesodermal and ectodermal origin, especially the cuticular epidermis (Momoyama et al., 1994; Richman et al., 1997). Haemocytic encapsulations are common in tissues undergoing necrosis due to WSSV infections. A number of groups have developed in situ hybridization and PCR diagnostic tests (Durand et al., 1996; Lo et al., 1996; Takahashi et al., 1996; Wongteerasupaya et al., 1996).

WSSV-infected nuclei are hypertrophic, have marginated chromatin, and contain single lightly eosinophilic to deeply basophilic inclusions (Momoyama et al., 1994; Wongteerasupaya et al., 1995; Durand et al., 1997; Richman et al., 1997). Rod-shaped virions are scattered throughout the granular viroplasm with membrane fragments, and accumulate at the inner nuclear membranes (Fig. 1). Paracrystalline arrays of WSSV have been observed (Inouye et al., 1994). The virus has not been reported in the cytoplasm of nonlytic cells.



**Figure 1.** White spot syndrome virus (WSSV) in the nucleus of a gill epithelial cell of an experimentally infected *Litopenaeus vannamei*. Note the rod-shaped virions (arrows) within viroplasm (V). Scale bar = 590 nm. Uranyl acetate and lead citrate. (Electron microscopy by Rena Krol.)

#### 2.2.4. Pathogen viability

A number of studies have shown that WSSV is relatively susceptible to inactivation by heating. Semipurified WSSV is inactivated within 20 min at 50°C, 1 min at 60°C and 0.2 min at 70°C (Chang et al., 1998; Maeda et al., 1998; Nakano et al., 1998). WSSV in tissue may be somewhat more resistant to heating. WSSV remains viable in frozen prawn tissue for an extended period. Nunan et al. (1998) were able to transmit WSSV to susceptible prawns by injecting cell-free extracts from prawn tails displaying clinical signs of white spot disease which were on sale in a supermarket in the USA.

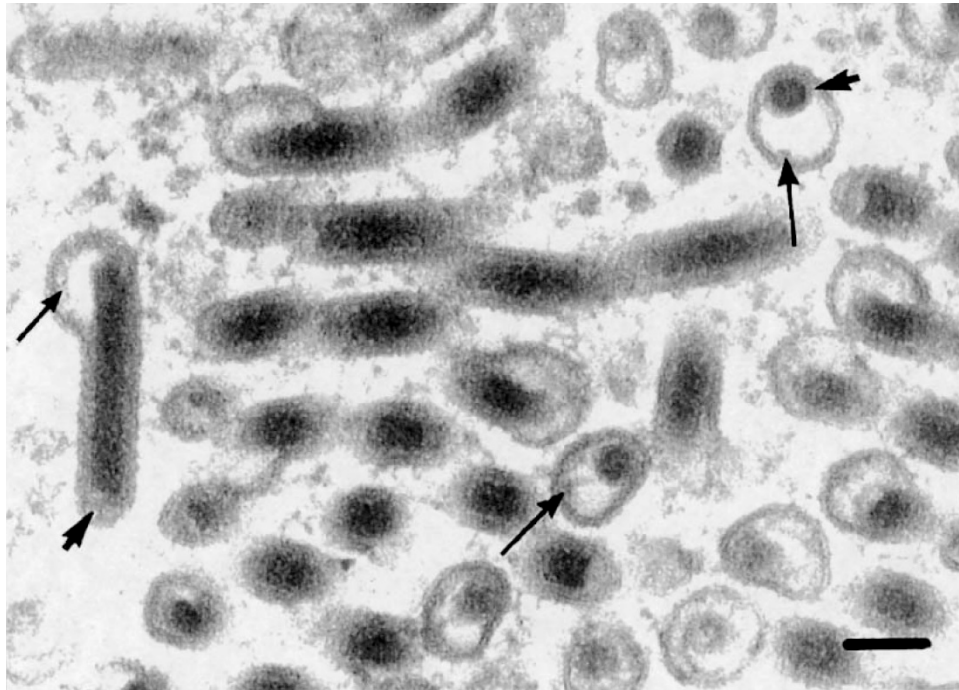
WSSV in sterile seawater kept at 30°C and in the dark remains viable for up to 30 days (Momoyama et al., 1998; Maeda et al., 1998) but is believed to be inactivated in about 3 days in prawn aquaculture ponds due to UV radiation and heating (Jory and Dixon, 1999).

Treatments to achieve complete inactivation have been determined for the following: chlorine compounds, formalin, povidone iodine, ethyl alcohol, ozone, UV, and pH (Chang et al., 1998; Maeda et al., 1998; Nakano et al., 1998).

### 2.3. Condition: *Astacus bacilliform virus* (AaBV) infection

#### 2.3.1. Causative agent

AaBV virions are composed of a cylindrical nucleocapsid surrounded by a closely applied trilaminar envelope, which expands unilaterally to contain a reflexed tail-like structure that arises from the end of the nucleocapsid (Fig. 2) (Edgerton et al., 1996). In thin sections, virions are approximately  $340 \times 70$  nm, and nucleocapsids are approximately  $260 \times 50$  nm.



**Figure 2.** *A. astacus* bacilliform virus (AaBV) in hepatopancreatocytes in *A. astacus*. AaBV consists of a rod-shaped nucleocapsid with an electron-dense nucleoprotein core and less electron-dense capsid (short arrows) and which is surrounded by a closely applied trilaminar envelope with a unilateral expansion at one to contain a reflexed taillike structure (thin arrows). Scale bar = 91 nm. Uranyl acetate and lead citrate.

#### 2.3.2. Epizootiology

AaBV was discovered recently in *A. astacus* in central Finland (Edgerton et al., 1996). In that study, 15 adult crayfish were collected from each of five populations. AaBV infected 100% of *A. astacus* from four populations, and 53.3% of *A. astacus* from the remaining population. Nothing is known about the transmission or potential host range of AaBV.

#### 2.3.3. Pathology

AaBV was not associated with clinical disease. However, Edgerton et al. (1996) considered that the intensity of some infections was high, which prompted them to suggest that AaBV may be pathogenic to *A. astacus*.



AaBV infects all cell types in the hepatopancreatic tubule epithelium, midgut and midgut caecum epithelial cells, and tegmental gland cells at the junction of the midgut and hindgut (Edgerton et al., 1996). AaBV often infects a high proportion of cells in the hepatopancreas, midgut and midgut caecum. Heavily infected epithelia are often necrotic and encapsulated by haemocytes.

Cells infected by AaBV have mildly hypertrophic, irregular-shaped nuclei (Edgerton et al., 1996). Nuclei have marginated chromatin and contain amorphous eosinophilic inclusions that are often darker toward the center, haloed, or compartmentalized. The nucleolus is dissolved in late infections, and the cytoplasm may be deeply basophilic. Rod-shaped virions are scattered or arranged in rows within a granular viroplasm in the nucleus. Virions are only present in the cytoplasm of infected cells following nuclear lysis.

#### 2.3.4. *Pathogen viability*

Nothing is known about the viability of AaBV.

### 2.4. *Condition: picorna-like virus infection in A. astacus*

#### 2.4.1. *Causative agent*

A picorna-like virus, approximately 20 nm in diameter, was observed in gill connective tissue in *A. astacus* (Halder, 1987). There is no other information on this virus in the current literature.

### 2.5. *Condition: Pacifastacus leniusculus bacilliform (PIBV) virus infection*

#### 2.5.1. *Causative agent*

PIBV virions are rod shaped and enveloped, and are 240 × 66 nm. Nucleocapsids are 189 × 44 nm.

#### 2.5.2. *Epizootiology*

PIBV has been reported on one occasion in *Pacifastacus leniusculus* in USA (Hedrick et al., 1995). The virus is yet to be properly described. Nothing is known about the transmission or potential host range of PIBV.

#### 2.5.3. *Pathology*

No signs of infection have been documented for PIBV, and it is unknown whether it causes disease. PIBV infects cells in the hepatopancreatic tubule epithelium (Hedrick et al., 1995). Infected cells may detach from the hepatopancreatic tubule basement membrane.

PIBV-infected cells have hypertrophic nuclei with marginated chromatin, which contain a granular eosinophilic inclusion. PIBV accumulates in the nucleus.

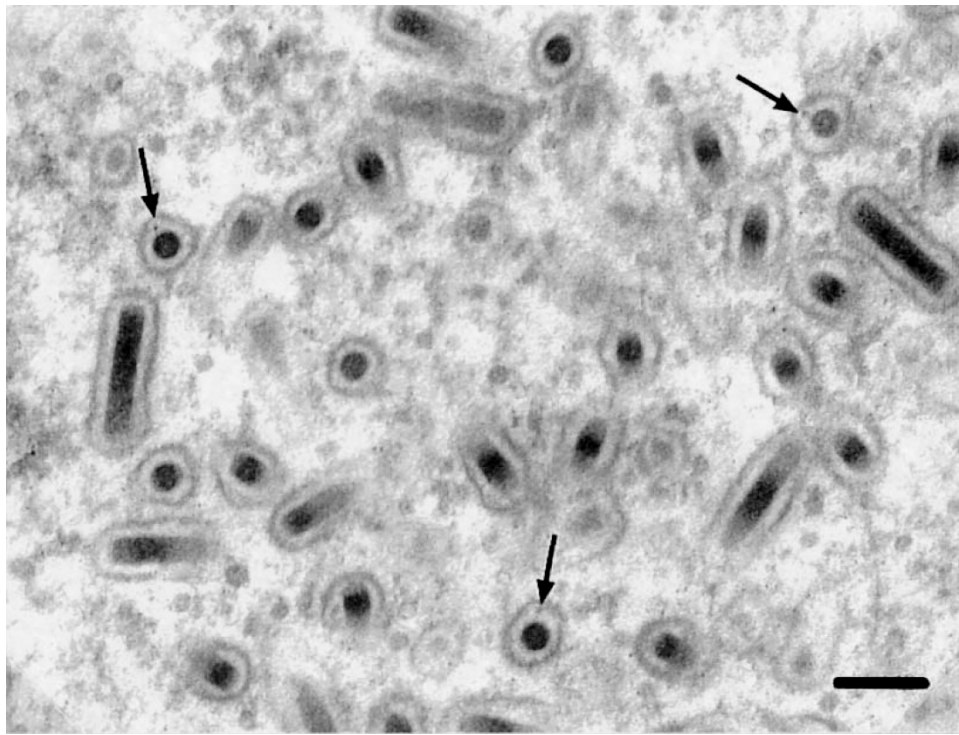
#### 2.5.4. *Pathogen viability*

Nothing is known about the viability of PIBV.

## 2.6. Condition: *Cherax quadricarinatus* bacilliform virus (CqBV) infection

### 2.6.1. Causative agent

CqBV consists of a cylindrical nucleocapsid surrounded by a loose envelope (Anderson and Prior, 1992; Groff et al., 1993; Edgerton, 1996a,b). The envelope is unilaterally expanded to contain a flexed tail-like structure that arises from one end of the nucleocapsid (Fig. 3). There is some disparity about the size of CqBV in the literature; Anderson and Prior (1992) found that CqBV was approximately  $200 \times 70$  nm, and nucleocapsids were  $150 \times 30$  nm. However, Groff et al. (1993) and Edgerton (1996a,b) agreed that virions were approximately  $260 \times 100$  nm, and nucleocapsids were  $215 \times 50$  nm. Edgerton (1996a,b) suggested that there may be several strains of CqBV corresponding to the many strains of *C. quadricarinatus*, but could not rule out differences in fixation causing the different morphometrics.



**Figure 3.** *C. quadricarinatus* bacilliform virus (CqBV) in hepatopancreatocytes in *C. quadricarinatus*. CqBV consists of a rod-shaped nucleocapsid surrounded by a loosely applied trilaminar envelope that has a slight unilateral bulge to accommodate a reflexed tail-like structure (arrows) at one end. Scale bar = 150 nm. Uranyl acetate and lead citrate.

### 2.6.2. Epizootiology

CqBV was the first virus found naturally infecting a freshwater crayfish (Anderson and Prior, 1992). CqBV infects both wild and farmed *C. quadricarinatus* in northern Queensland and the Northern Territory, Australia (Anderson and Prior, 1992). It has also been introduced into the USA (Groff et al., 1993) and Ecuador (Xavier Romero, pers. comm.). CqBV

infected up to 70.5% of *C. quadricarinatus* in six of seven northern Queensland farms sampled in two histopathological surveys (Edgerton, 1996a,b; Edgerton et al., 1995; Edgerton and Owens, 1999). CqBV has been associated with mortalities in experimental and aquacultured populations of *C. quadricarinatus* (Edgerton and Owens, 1997; Edgerton et al., 1995). It is, however, considered to have low virulence to *C. quadricarinatus* as epizootic mortalities have not been observed in farms that have a high prevalence of CqBV (Edgerton, 1996a; Edgerton and Owens, 1999).

CqBV has been observed in all life stages of *C. quadricarinatus* after juvenile stage 3. Juvenile *C. quadricarinatus* are first susceptible to CqBV soon after molting into stage 3, that is, the first feeding stage (Edgerton and Owens, 1997). This was considered proof that CqBV was transmitted per os possibly within fecal strings, attached to detrital particles, or associated with phytoplankton or zooplankton.

The host range of CqBV is unknown.

### 2.6.3. Pathology

*C. quadricarinatus* with heavy CqBV infections are lethargic, have a weakened or failed tail-flick response, and are unable to right themselves when placed on their back (Edgerton, 1996a,b).

CqBV infects the relatively senescent proximal hepatopancreatocytes (Anderson and Prior, 1992; Groff et al., 1993), as well as the epithelial cells of the antechamber, midgut, and proximal midgut caecum (Edgerton, 1996a,b). Bacteraemia is common in *C. quadricarinatus* with intense CqBV infections (Edgerton, 1996a,b). Necrosis of the hepatopancreatic tubule epithelium, and hepatopancreatic atrophy have been observed in a few acute cases (Edgerton, 1996a,b).

CqBV infection causes marked nuclear changes, including hypertrophy, chromatin, and nucleolar margination, and the formation of single, granular, eosinophilic inclusions (Anderson and Prior, 1992; Groff et al., 1993; Edgerton, 1996b). Infected nuclei therefore have a typical signet ring appearance. Fine strands of chromatin may compartmentalize the inclusion, and fixation may result in shrinkage of the inclusion, giving it a haloed appearance. The cytoplasm may have increased basophilia. Early infections are characterized by small, haloed, eosinophilic intranuclear inclusions in susceptible cells.

CqBV accumulates in the nucleus within a granular viroplasm (Anderson and Prior, 1992; Groff et al., 1993; Edgerton, 1996a,b). Rounded putative nucleolar remnants are common within nuclei (Anderson and Prior, 1992; Edgerton, 1996a,b). Anderson and Prior (1992) reported long tubular structures in the viroplasm; however, these were not observed by Edgerton (1996b) or reported by Groff et al. (1993).

### 2.6.4. Pathogen viability

Nothing is known about the viability of CqBV.

## 2.7. Condition: *Cherax* *Giardiavirus-like virus* (CGV) infection

### 2.7.1. Causative agent

CGV is icosahedral, nonenveloped, and is approximately 25 nm in diameter (Edgerton et al., 1994).

### 2.7.2. Epizootiology

CGV was first observed in *C. quadricarinatus* collected in a histopathological survey of crayfish collected from farms in northern Queensland, Australia (Edgerton et al., 1994, 1995). It was subsequently associated with mortalities in experimental juvenile *C. quadricarinatus* populations (Edgerton et al., 1994). Infection studies showed that the prevalence of CGV rose dramatically amongst juveniles following molting into stage 3 (58% and 86% at 3 and 6 weeks post-juvenile stage 3, respectively), and that the intensity of infections in juveniles was often very high (Edgerton and Owens, 1997). CGV is very common in farmed redclaw in northern Queensland (Edgerton et al., 1995; Edgerton, 1996a).

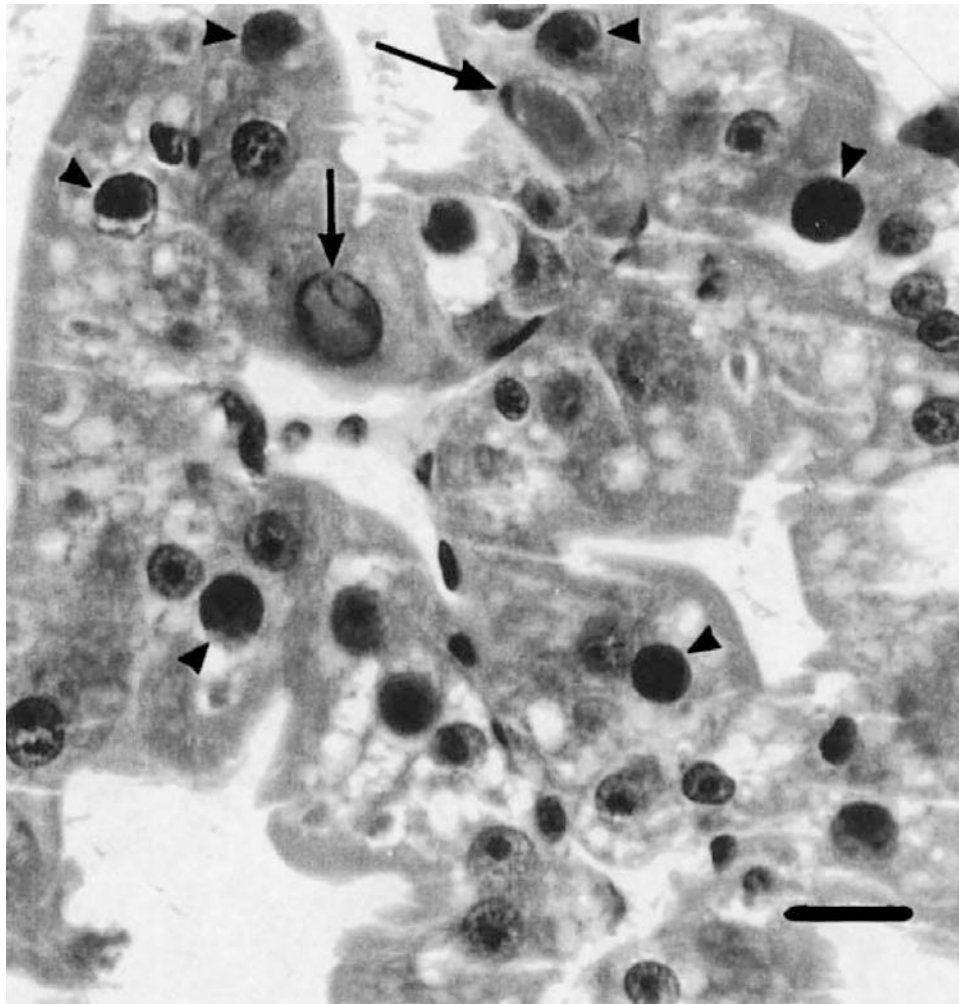
Edgerton and Owens (1997) showed that *C. quadricarinatus* is first susceptible to infection by CGV immediately after molting into juvenile stage 3, which is the first feeding stage. These authors considered this to be proof that CGV is transmitted per os possibly within fecal strings, attached to detrital particles, or associated with phytoplankton or zooplankton.

### 2.7.3. Pathology

Current information suggests that CGV may not cause disease in adult *C. quadricarinatus* (Edgerton, 1996a). Intense infections may, however, result in morbidity in juvenile *C. quadricarinatus* (Edgerton et al., 1994).

CGV has been observed only in hepatopancreocytes, most commonly in R cells (Edgerton et al., 1994). F and B cells are also infected. However, E cells never show signs of infection by either electron or light microscopy. CGV-infected cells do not usually slough into the hepatopancreatic tubule lumen.

CGV infection causes negligible to mild nuclear hypertrophy (Edgerton et al., 1994). Infected nuclei have margined and clumped chromatin, and contain multiple purple-red staining (with H&E) inclusions that are entirely composed of virions in paracrystalline arrays. In late infections, the inclusions coalesce and so become fewer in number and larger. The nucleolus remains identifiable and is usually central, sometimes within a rosette of inclusions (Fig. 4).



**Figure 4.** Juvenile *C. quadricarinatus* with intense infections by *Cherax Giardavirus*-like virus and *Cherax quadricarinatus* bacilliform virus. Scale bar = 25  $\mu$ m. H&E.

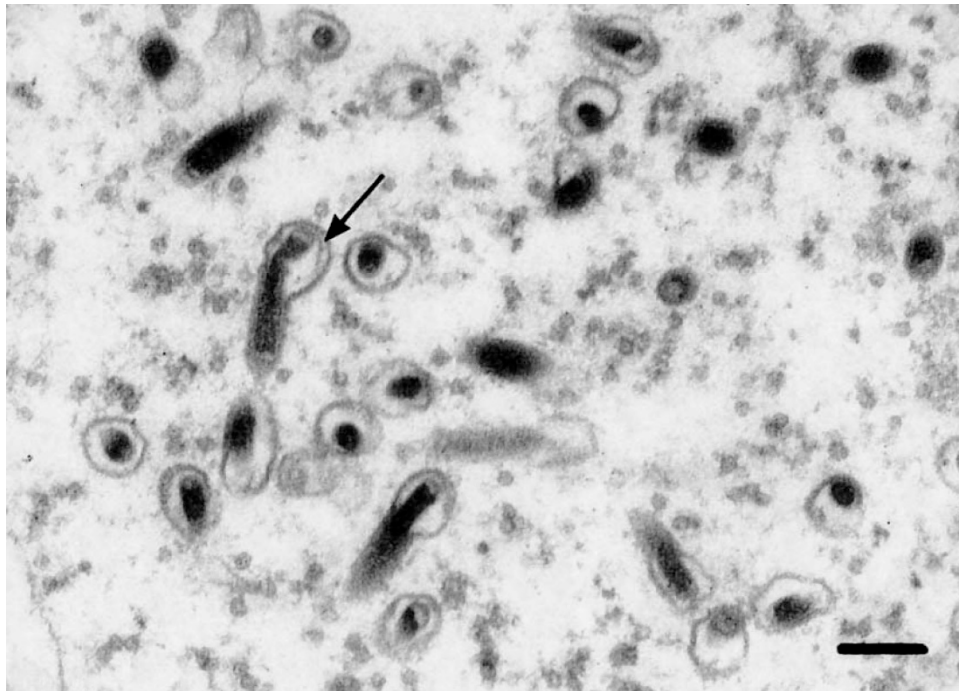
#### 2.7.4. Pathogen viability

Nothing is known about the viability of CGV.

### 2.8. Condition: *Cherax destructor bacilliform virus* (CdBV) infection

#### 2.8.1. Causative agent

CdBV consists of a cylindrical nucleocapsid that is slightly to markedly bent within a trilaminar envelope (Edgerton, 1996a,b). The envelope expands laterally in the bent region and accommodates a tail-like structure that arises from that end of the nucleocapsid (Fig. 5). Virions are approximately  $260 \times 100$  nm and nucleocapsids are approximately  $210 \times 50$  nm. Several long enveloped nucleocapsids were also observed.



**Figure 5.** *Cherax destructor* bacilliform virus (CdBV) in hepatopancreatocytes in *C. destructor*. CdBV consists of a rod-shaped nucleocapsid surrounded by a closely applied trilaminar envelope that is expanded at one end to accommodate a reflexed tail-like structure (arrows). Note that virions are often bent at the end with the tail-like structure. Scale bar = 150 nm. Uranyl acetate and lead citrate.

### 2.8.2. Epizootiology

CdBV infected 1 crayfish from northwestern South Australia, and 3 of 9, and 1 of 14 crayfish collected from a farm in southeastern South Australia (Edgerton, 1996a,b). Nothing more is known about its prevalence, transmission or potential host range.

### 2.8.3. Pathology

The one study on CdBV was too limited to assess whether it caused disease. CdBV infects hepatopancreatocytes and infection results in lysis and sloughing (Edgerton, 1996a,b). CdBV-infected nuclei are markedly hypertrophic, have marginated chromatin and contain a single amorphous eosinophilic inclusion, corresponding to a granular viroplasm (Edgerton, 1996a,b). Virions are scattered throughout the viroplasm and accumulate at the nuclear membrane. The viroplasm contains abundant putative ribosomal precursor particles and membrane fragments that often form circles. The cytoplasm of infected cells often contains increased basophilia. In late infections, the cytoplasm contains large vesicles formed by dilation of endoplasmic reticulum cisternae (Edgerton, unpublished data) and abundant free ribosome, and the plasma membrane becomes detached from the basement membrane of the hepatopancreatic tubule. Cell-to-cell transmission is presumed to occur following lysis of cells, as virions have not been observed in the cytoplasm.

2.8.4. *Transmission and viability*

Nothing is known about the viability of CdBV.

2.9. *Condition: Cherax destructor systemic parvo-like virus (CdSPV) infection*

2.9.1. *Causative agent*

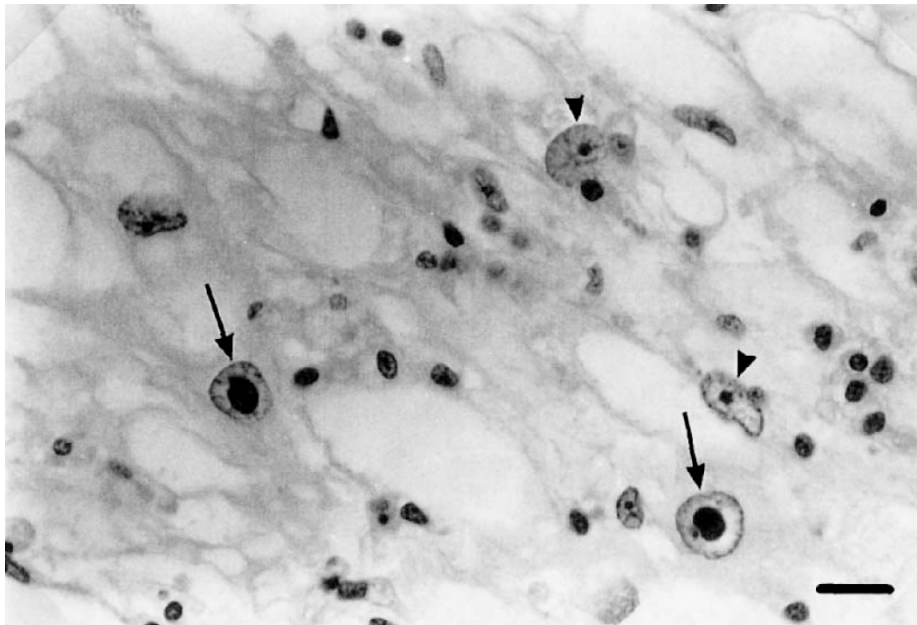
CdSPV particles are icosahedral and approximately 21 nm (Edgerton et al., 1997).

2.9.2. *Epizootiology*

CdSPV has been observed in only one *C. destructor* from a farm in southeastern South Australia (Edgerton, 1996a; Edgerton et al., 1997). No other studies have been done; hence the distribution and prevalence of this virus is not known.

2.9.3. *Pathology*

The one CdSPV-infected *C. destructor* was morbid when collected from a pond bank (Edgerton, 1996a; Edgerton et al., 1997). Patches of opaque musculature were observed through the translucent cuticle on the ventral surface of the abdomen. This crayfish exhibited extensive necrosis in several major organs including the gills, hepatopancreas, and muscle. However, the involvement of CdSPV in these lesions was unclear as gill was the only tissue in which the cytopathic lesions were common. Infected cells were also observed in the epicardium and spongy connective tissues (Fig. 6).



**Figure 6.** *Cherax destructor* systemic parvo-like virus (CdSPV) intranuclear Cowdry type A inclusions (arrows) in the epicardium in *C. destructor*. Note also other hypertrophic nuclei without inclusions (arrowheads), although one may be developing an inclusion. Scale bar = 25  $\mu$ m. H&E.

Nuclei of CdSPV-infected cells were markedly hypertrophic, had marginated chromatin and contained Cowdry type A intranuclear inclusions (Edgerton, 1996a; Edgerton et al., 1997). Inclusions consisted predominantly of empty capsids and microfilaments. Aggregates of complete viral particles were observed outside of the inclusion. Nucleoli were often hypertrophic and their fibrous and granular components were segregated; the fibrous component was vacuolated and was surrounded by the granular component. One nucleus, presumed to be in a very early stage of infection, contained an altered nucleolus and a developing, rounded viroplasm that consisted primarily of long microfilaments with associated empty capsids.

#### 2.9.4. *Transmission and viability*

Nothing is known about the transmission, potential host range, or viability of CdSPV.

#### 2.10. *Concluding remarks about freshwater crayfish viruses*

The relationship between infection and disease remains obscure for the majority of crayfish viruses. For some of the better studied crayfish viruses, such as CqBV and CGV, circumstantial evidence suggests that disease, or at least large-scale mortalities, may be prevented in aquaculture situations by proper husbandry (Edgerton, 1996a). However, there is evidence to suggest that CGV is capable of causing disease and mortalities in juvenile *C. quadricarinatus*. Recent occurrence of virus-induced epizootics in prawn aquaculture in most prawn-growing countries underlines the threat that introduced viruses represent to crustacean aquaculture and conservation.

### 3. **Rickettsiales**

#### 3.1. **Condition: systemic rickettsial infection**

##### 3.1.1. *Causative agents*

The RLOs are rod shaped in appearance and had a mean length of 0.5  $\mu\text{m}$  and width of 0.16  $\mu\text{m}$  (Ketterer et al., 1992). Slightly smaller dimensions were reported for RLO found in *C. quadricarinatus* reared in Ecuador (0.3  $\mu\text{m}$  length and 0.1  $\mu\text{m}$  width; Jiménez et al., 1997).

##### 3.1.2. *Epizootiology*

Gram-negative, intracellular, prokaryotic organisms called rickettsia-like or members of the Rickettsiales have been described infecting cells of a number of aquatic crustaceans including freshwater amphipods (Federici et al., 1974), marine crabs (Johnson, 1984; Bonami and Pappalardo, 1980), isopods (Vago et al., 1970), and penaeid shrimps (Lightner et al., 1985; Brock et al., 1986; Anderson et al., 1987). There have been five reports of this type of organism in freshwater crayfish, all described in *C. quadricarinatus*. Affected animals were obtained from crayfish ponds in Queensland (Ketterer et al., 1992; Owens et al., 1992; Edgerton et al., 1995; Edgerton, 1996) and in Ecuador (Jiménez et al., 1997).



Rickettsial infections in freshwater crayfish have been demonstrated in moribund animals collected during investigation of mortalities in crayfish ponds. In one study, a mortality of 22% was reported and the mortality rate significantly increased when animals were transferred from one pond to another (Ketterer et al., 1992). In Ecuador, the mortality rate ranged from 45% to 80% (Jiménez et al., 1997). In another investigation in Queensland, a chronic, low-grade mortality was observed and 10 of the 32 moribund animals examined were infected with systemic RLOs (Edgerton et al., 1995).

There is no report on transmission of systemic RLOs to crayfish. Successful transmission through inoculation of healthy crabs, *Carcinus metapenaeus*, with tissue suspensions of diseased crabs has been reported (Bonami and Pappalardo, 1980). Feeding tissues of infected animals to healthy animals of the same or similar species has also been shown to reproduce a rickettsia-like disease in the terrestrial sow bug *Armadillidium vulgare* (Vago et al., 1970).

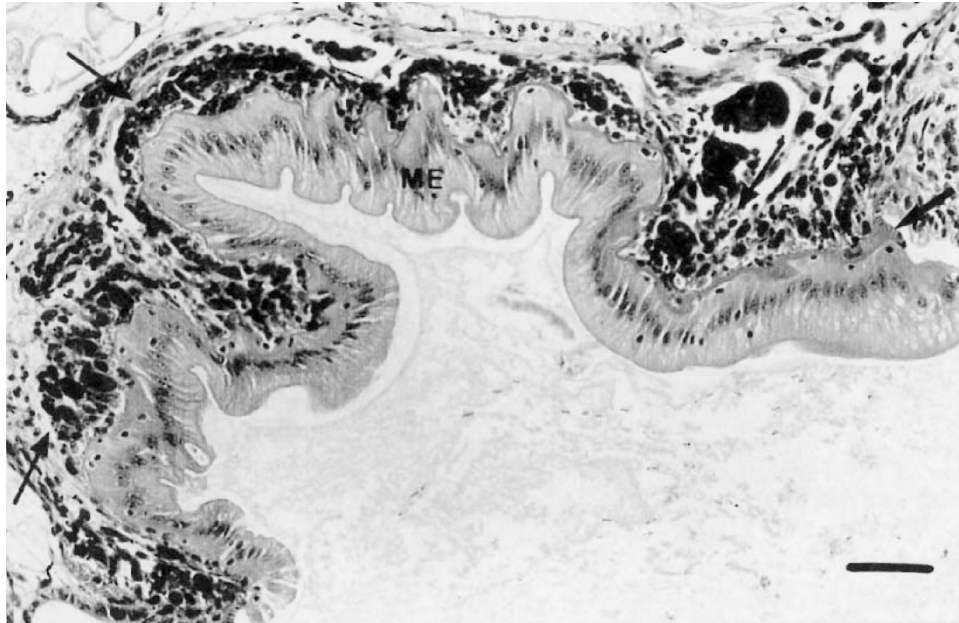
### 3.1.3. Pathology

Crayfish infected with systemic RLOs were weak and exhibited a weakened tail-flick response and an inability to right themselves when laid on their back (Edgerton et al., 1995). In one study, all crayfish infected with systemic RLOs were a “mottled bluish” color (Edgerton, 1996). A blue coloration was also noted in some of the infected crayfish in Ecuador (Jiménez et al., 1997).

Histopathological examination revealed cytoplasmic, basophilic Gram-negative microcolonies of RLOs infecting the connective tissue of all organ systems and endothelia of hemolymph vessels. Infected cells were hypertrophied and tissues showed hyperplasia. Tissue necrosis was frequently observed as was atrophy of the hepatopancreas. The tropism of RLOs was similar in four studies. The gills, heart, eyes, neural tissue, nephridial canal of the antennal gland, and hemocytes can all become intensely infected (Ketterer et al., 1992; Owens et al., 1992; Edgerton et al., 1995; Edgerton 1996). Infected cells typically display a cytoplasmic, basophilic inclusion body (Fig. 7) with dense aggregates of RLOs contained within a thin-walled vacuole.

### 3.1.4. Pathogen viability

There is no report on the viability of the systemic RLOs.



**Figure 7.** Microcolonies of the systemic rickettsia-like organism (thin arrows) in the submucosa of the midgut in *C. quadricarinatus*. Note that the midgut epithelium (ME) is not infected, and that the basement membrane of the midgut epithelium has thickened (thick arrow). Scale bar = 94  $\mu$ m. H&E.

### **3.2. Condition: hepatopancreatic rickettsial infection**

#### **3.2.1. Causative agent**

The hepatopancreatic rickettsia-like organism (RLO) is pleomorphic, with rounded forms more common than rod-shaped forms, and 0.2–0.4  $\mu$ m in length (Edgerton and Prior, 1999).

#### **3.2.2. Epizootiology**

The RLO was observed in one moribund *C. quadricarinatus* in north Queensland (Edgerton and Prior, 1999). There have been no further reports of this RLO, and nothing is known about its transmission or potential host range.

#### **3.2.3. Pathology**

The hepatopancreatic RLO can be differentiated from the systemic RLO on the basis of tissue tropism; as the name suggests, hepatopancreatic RLO is restricted to the hepatopancreatic tubule epithelium, whereas the systemic RLO has never been observed in these tissues (Edgerton and Prior, 1999). Basophilic, Gram-negative inclusions are observed in the cytoplasm of infected cells by light microscopy, and transmission electron microscopy reveals that the inclusions consist of rod-shaped RLOs microcolonies.

#### **3.2.4. Pathogen viability**

Nothing is known about the viability of the hepatopancreatic RLO.

## 4. Bacteria

### 4.1. Condition: asymptomatic bacteremia and bacterial septicemia (called vibriosis if *Vibrio* species are predominant pathogen)

#### 4.1.1. Causative agents

Bacterial species from numerous genera including both Gram-negative (*Acinetobacter*, *Aeromonas*, *Citrobacter*, *Flavobacterium*, *Pseudomonas*, and *Vibrio*) and Gram-positive (*Corynebacterium*, *Bacillus*, *Micrococcus*, and *Staphylococcus*) species.

#### 4.1.2. Epizootiology

Asymptomatic bacteremia have been reported in apparently healthy freshwater crayfish (Scott and Thune, 1986; Wong et al., 1995; Webster, 1995; Madetoja and Jussila, 1996). It is characterized by the presence of a mixed bacterial population in hemolymph samples collected under aseptic conditions and cultured in appropriate media, usually blood or nutrient agar. In all reported studies, the crayfish were collected from crayfish culture ponds or tanks and histopathology was not performed on the sampled stock. However, in the case of one study (Webster, 1995), histopathological investigations performed on other crayfish collected from the same farm revealed high prevalence of bacteremic lesions (Edgerton et al., 1995).

This condition has been demonstrated in numerous crayfish species including *A. astacus*, *C. destructor albidus*, *C. quadricarinatus*, and *Procambarus clarkii*. Both Gram-negative and Gram-positive bacteria have been isolated from crayfish hemolymph. The most frequently reported Gram-negative genera are *Pseudomonas*, *Aeromonas*, *Acinetobacter*, *Flavobacterium*, and *Vibrio micrococcus* and *Staphylococcus* are the most often reported Gram-positive genera (Scott and Thune, 1986; Wong et al., 1995; Madetoja and Jussila, 1996).

One study (Scott and Thune, 1986) reported 9.3% of crayfish with total counts greater than 50 bacteria per milliliter of hemolymph. Other workers (Wong et al., 1995) reported counts of greater than 100 bacteria per milliliter in approximately 50% of the test animals. Gram-negative rods were the predominant bacterial group comprising 50% of bacterial species observed in a study on *A. astacus* (Madetoja and Jussila, 1996), 35% in *C. quadricarinatus* and 77% in *C. destructor albidus* (Wong et al., 1995). The prevalence of asymptomatic bacteremia in apparently healthy farmed or laboratory-held animals varied from 41% to 100% (Scott and Thune, 1986; Wong et al., 1995; Webster, 1995; Madetoja and Jussila, 1996).

The etiology of asymptomatic bacteremia in freshwater crayfish and its pathological significance is unclear. Studies on other crustacean species suggest that bacteria are not normally present in crustacean hemolymph (Bang, 1970; Lee and Pfeifer, 1975; Johnson, 1976). However, bacteria have been isolated from the hemolymph of apparently healthy spiny lobsters (Brinkley et al., 1976), crabs (Colwell et al., 1975; Welsh and Sizemore, 1985), penaeid shrimp (Lightner, 1977), and other crustacean species (Bang, 1970) as well as from apparently healthy crayfish.

Most authors suggest that the presence of bacteria in the hemolymph of healthy crustaceans is the result of exposure to environmental stressors. Studies in freshwater crayfish tend to support this claim. For example, the prevalence of bacteremia in healthy, pond-

reared crayfish has been shown to increase during periods of elevated temperature and low dissolved oxygen (Scott and Thune, 1986; Thune, 1994). Increasing time of holding in laboratory tanks under suboptimal conditions has also shown to result in an increase in the prevalence of bacteremia in test animals (Madetoja and Jussila, 1996). It is of significance that sampling of hemolymph for bacterial culture in apparently healthy animals is usually conducted after the crayfish have been transported to the laboratory and, possibly, held in air or in aquaria for extended periods. Such procedures are likely to evoke stress responses in the test animals which could lead to the development of bacteremia. Thus, prevalence of this condition in healthy crayfish may be frequently overestimated.

Bacterial septicemia is characterized by the presence of gross clinical signs such as lethargy, reduced response to stimuli, loss of muscle tone or postural abnormalities, bacteremia and histopathological lesions such as small nodules or granulomas, hemocyte aggregations, and encapsulation reactions. In mild cases, clinical signs are lacking and the condition is diagnosed through histopathological examination. The presence of clinical and/or histopathological disease signs distinguishes this condition from asymptomatic bacteremia. However, the two conditions are probably both bacterial infections, the latter representing an early phase of the former.

Bacterial septicemia is mostly described as an opportunistic infection whereby mildly pathogenic strains of ubiquitous bacteria enter the haemocoel through the oral route or through wounds, proliferate in the hemolymph and then multiply in body tissues (Vey et al., 1975; Johnson, 1983; Alderman and Polglase, 1988). Freshwater crayfish displaying this condition have mostly been obtained through collection of moribund animals from wild populations (Toumanoff, 1965, 1966, 1967, 1968; McKay and Jenkin, 1969; Vey et al., 1975; Boemare and Vey, 1977) or farm ponds (Thune et al., 1991; Edgerton et al., 1995). Animals have also been collected as part of health surveys (Evans et al., 1992; Edgerton et al., 1995) and from infectivity trials (Amborski et al., 1975; Roy, 1993; Wong et al., 1995). Two studies of natural infections in farm ponds (Thune et al., 1991; Eaves and Ketterer, 1994) reported high levels of mortality in the crayfish populations under investigation.

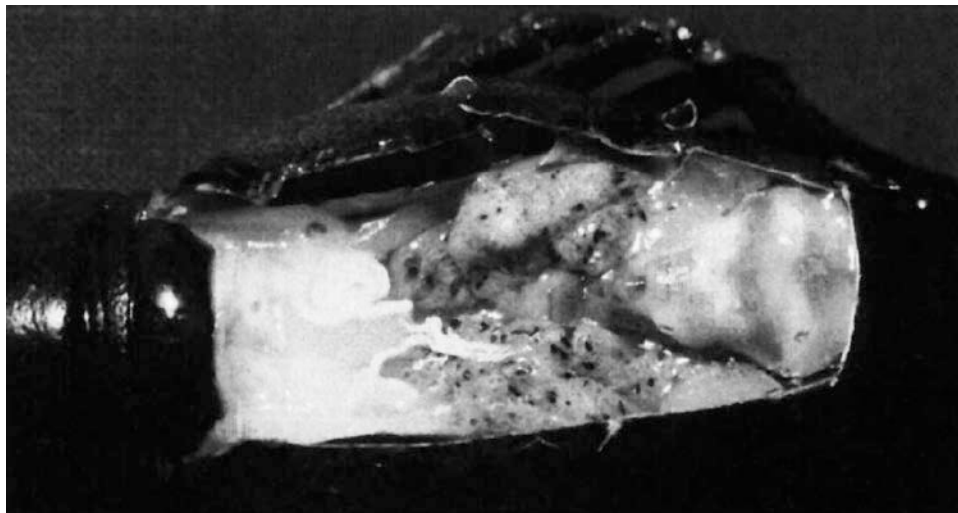
A number of different species of bacteria have been isolated from affected crayfish but *Pseudomonas* sp. (McKay and Jenkin, 1969), *Pseudomonas morganii*, *Pseudomonas aerogenes*, and *Proteus vulgaris* (Toumanoff, 1965, 1966, 1967, 1968), *Pseudomonas florescens*, and *Pseudomonas putida* (Vey et al., 1975), *Aeromonas hydrophila* (Edgerton et al., 1995) and *V. mimicus* and *V. cholerae* (Thune et al., 1991) have been consistently isolated from crayfish displaying gross clinical features of bacterial septicemia.

Of particular interest was the study by Thune et al. (1991) of moribund crayfish collected from various crayfish holding and culture systems during epizootics occurring near the end of the harvest season. These events were characterized by periods of elevated temperatures and reduced dissolved oxygen levels. A total of 15 cases were studied, 5 from soft-shell operations, 5 from purging systems, 4 from ponds, and 1 from a holding tank. *V. mimicus* and *V. cholerae* were the predominant organisms isolated from all case studies, and these isolates were found to express a single serotype. *V. mimicus* was the predominant bacterial species in another study of mortalities in crayfish ponds (Eaves and Ketterer, 1994) and has been shown to cause rapid mortality when used in infectivity trials (Wong et al., 1995).

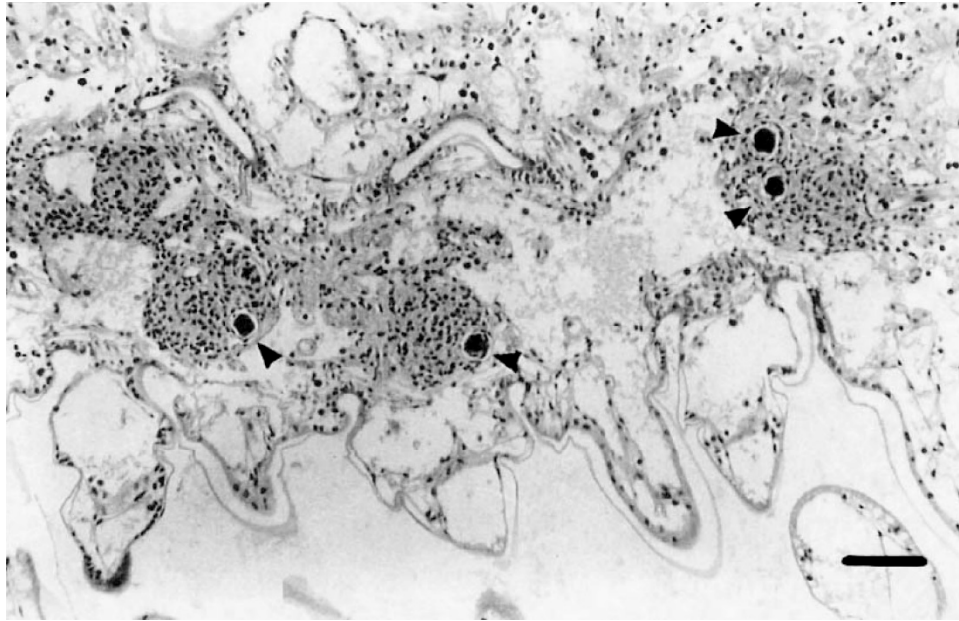
Bacteria found in freshwater crayfish inhabit the ecosystem in which the crayfish live and may be found in water and sediments, and reside on the exoskeleton or in the gut. Evidence from studies with the blue crab suggest that transmission is via the aquatic environment with bacteria gaining entry to the haemocoel through minor wounds, the gastrointestinal tract, or other routes (Tubiashi et al., 1975; Davis and Sizemore, 1982). Vey et al. (1975), using the pathogenic pseudomonads, *Pseudomonas florescens* and *Pseudomonas putida*, were able to induce bacterial disease by placing wounded crayfish in water containing the bacteria. Infections via the oral route were also achieved. Similarly, transmission through the aquatic environment was proposed by Wong et al. (1995) who demonstrated *V. mimicus* isolates of the same ribotype in pond water and hemolymph of apparently healthy crayfish from the same pond.

#### 4.1.3. Pathology

Crayfish with bacterial septicemia are typically lethargic and exhibit lack of muscle tone, reduced response to stimulus and a tendency to lie on their sides. Histopathology reveals host defense reactions in a number of different body organs (Figs. 8 and 9). Histological sections obtained from crabs (Johnson, 1976) and European crayfish species (Vey et al., 1975) showed the bacteria to be confined to the hemolymph, phagocytic hemocytes, and fixed phagocytes of the hepatopancreas. Bacteria were also occasionally seen in focal areas of necrotic muscle. As the disease progressed, hemocyte aggregations were seen in the heart, hemal sinuses and spaces, hepatopancreas, gills, antennal gland, and the Y organ (Vey et al., 1975; Johnson, 1976).



**Figure 8.** Macroscopic view of hepatopancreas of *A. astacus* with enteric bacterial infection. Black spots are foci of melanization in response to infection. (Photograph by Birgit Oidtmann, University of Munich.)



**Figure 9.** Multiple nodules with continued haemocytic aggregation and whirling (arrow-heads) in the gills in *C. quadricarinatus* with bacteraemia. Scale bar = 130  $\mu\text{m}$ . H&E.

Histopathological studies on Australian freshwater crayfish with bacterial septicemia revealed perivascular cuffing of hepatopancreatic hemolymph vessels and granulocytic hemocyte aggregations in the heart, gills, hepatopancreas, antennal gland, abdominal muscle, and connective tissue (Evans et al., 1992; Edgerton et al., 1995). In the former study, bacteria were occasionally evident in the heart and abdominal muscle. Edgerton et al. (1995) did not observe bacteria or bacterial colonies in histological preparations but follow-up bacterial isolation of hemolymph from crayfish from the same pond revealed prolific growth of *Pseudomonas* sp.

#### 4.1.4. Pathogen viability

Bacteria present in crayfish products proposed for import can be eliminated by heat treatment and other forms of sterilization. Exposure times and temperatures required to destroy different bacterial species vary depending on the species (Hocking et al., 1997). *V. cholera*, for example, is sensitive to temperatures above 45°C. Survival under refrigeration ( $\leq 10^\circ\text{C}$ ) will also vary depending on the bacterial species. *V. cholera* survives from between 2 and 4 weeks under refrigeration and probably longer when frozen ( $-20^\circ\text{C}$ ) (Desmarchelier, 1997).

In an infection study with freshwater crayfish, the viability of four bacterial species previously isolated from aquarium water, fish, bullfrogs, and crayfish was tested. Washed cells from stationary phase broth cultures of the four organisms were found to be approximately 50% viable after 4 days of starvation in glass distilled water (Amborski et al., 1975).

Thus, while bacterial species found in asymptomatic bacteria will be destroyed by appropriate sterilization and disinfection procedures, the bacteria can withstand other handling procedures not specifically aimed at killing the organism.

Eight isolates of *V. mimicus*, obtained from the freshwater prawn *Macrobrachium malcolmsonii*, have been shown to be sensitive to the antibiotics chloramphenicol, gentamicin and trimethoprim-sultame-thoxazole (Chowdhury et al., 1987).

#### **4.2. Condition: enteric bacterial infection**

##### *4.2.1. Causative agents*

*Citrobacter freundii* and *Citrobacter* sp., *Acinetobacterium* sp. and *Pseudomonas* sp. also commonly isolated from infected animals.

##### *4.2.2. Epizootiology*

Mortalities associated with enteric bacteria from the crayfish gut have been described in several species of European and North American crayfish (Toumanoff, 1965, 1967, 1968; Vey et al., 1975; Boemare and Vey, 1977; Oidtmann and Hoffmann, 1999). Infectivity trials with a strain of *Citrobacter freundii* caused lesions in normal crayfish force-fed large inoculums, but overall infectivity of bacterial isolates was low (Boemare and Vey, 1977). The authors suggested that disease is only likely to occur when the host is stressed by unfavorable environmental conditions.

##### *4.2.3. Pathology*

In the studies conducted by European workers, large numbers of bacteria were observed in the lumen of the midgut and the hepatopancreas of moribund animals. Bacteria were usually absent from epithelial cells. When present, the bacteria were associated with a massive hemocytic reaction in which necrotic hepatopancreatic tubules were encapsulated with layers of hemocytes. Deposition of melanin pigments was rarely observed.

Histopathological lesions in midgut and hepatopancreatic tissues similar to those described by Toumanoff (1965, 1967, 1968), Vey et al. (1975), Boemare and Vey (1977), and Vogt and Rug (1996), but also involving melanization, have been observed in *C. quadricarinatus* (Edgerton, 1996; Edgerton and Owens, 1999), and, in a milder form, in *C. tenuimanus* and *C. destructor albidus* (Evans et al., 1992). Crayfish examined in these two studies were obtained from crayfish ponds in Queensland and Western Australia. In both studies, crayfish were collected as part of a health survey on crayfish culture ponds and no gross sign of disease or mortalities was reported.

##### *4.2.4. Pathogen viability*

Appropriate disinfection and sterilization procedures should destroy bacteria causing this condition.

### **4.3. Condition: nocardiosis**

#### *4.3.1. Causative agent*

The *Nocardia* sp. had branched filaments within the nodules, 0.5–1.0  $\mu\text{m}$  in width and 10–15  $\mu\text{m}$  in length. They were found to be Gram-positive and acid fast.

#### *4.3.2. Epizootiology*

This condition has been described only in a single adult specimen of the white-clawed crayfish, *Austropotambius pallipes*, collected from the River Avon in England during an investigation of a crayfish plague outbreak (Alderman et al., 1986). Nothing is known about transmission of the *Nocardia* sp.

#### *4.3.3. Pathology*

The animal was sluggish and uncoordinated and, unusually for a nocturnal species, was found in daylight, out in the open on top of a weed bed. A depressed, unhealed fracture was present in the abdomen, and underlying tissues contained numerous small, spherical black nodules (0.5–2.0 mm diameter) scattered through the musculature. Histopathological examination revealed branched filaments surrounded by concentric layers of melanized host hemocytes. Filaments were not obvious when stained with H&E but were well revealed with combined Grocott methenamine silver and hematoxylin-eosin stain.

#### *4.3.4. Pathogen viability*

No information is available on viability.

### **4.4. Condition: bacterial cuticular fouling**

#### *4.4.1. Causative agents*

Filamentous *Leucothrix*-like bacteria. *Leucothrix mucor*, a marine species, is a septate filamentous bacterium, above 2  $\mu\text{m}$  in diameter of variable length and with a terminal gonidia (Johnson, 1983). Freshwater species are likely to display similar morphology.

#### *4.4.2. Epizootiology*

Filamentous bacteria have been described as epibionts of North American crayfish (Johnson, 1977) and of Australian crayfish (Evans et al., 1992; Edgerton and Owens, 1999). Filamentous bacteria were observed on the exoskeleton and in the gills. In these studies, the organisms were called filamentous bacteria and no taxonomic details or morphometric measurements were provided. Heavy infestations of gills by filamentous bacteria sometimes occur in marine crustaceans (see review, Johnson, 1983). The organisms form a thick mat on eggs and gills, interfering with respiration and other metabolic processes. Entanglement of larvae by the long filaments can interfere with swimming and molting. Heavy mortality can sometimes occur in these species (Johnson, 1983). However, excessive bacterial gill or exoskeleton fouling has not been reported in freshwater crayfish.

When present in marine aquaculture facilities, filamentous bacteria can proliferate under conditions of poor water quality (Solangi et al., 1979). Transmission of *Leucothrix* through



the aquatic environment has been reported (Johnson et al., 1971). Information on transmission of freshwater filamentous bacteria is lacking.

#### 4.4.3. Pathology

Wet mount microscopy on pleopod or gill filament preparations reveals characteristic bacterial filaments.

#### 4.4.4. Pathogen viability

Infestations with filamentous bacteria can be controlled using antibiotics (Johnson, 1983; Alderman and Polglase, 1988). Maintenance of good water quality (low nutrient load, high oxygen levels) is a well-known control measure.

## 5. Fungi

### 5.1. Condition: crayfish plague, *aphanomyiasis*, *la peste*, *krebspest*, *kraftpest*

#### 5.1.1. Causative agent

*Aphanomyces astaci*.

#### 5.1.2. Life cycle/life history

Factors affecting the growth and zoospore production of *Aphanomyces astaci* have been studied extensively (Unestam, 1965, 1966, 1969b). Large numbers of zoospores are released from zoosporangia when an infected crayfish is moribund or has recently died. These zoospores are motile for up to 3 days in water at 10°C and for shorter periods of time at higher water temperatures (Svensson, 1978). The zoospores can survive for up to 2 weeks in mud (Rennerfelt, 1936). The survival time is strain and temperature dependent (Diéguez-Uribeondo et al., 1995).

Zoospores appear to be chemotactically attracted to crayfish and often settle on the cuticle near a wound (Svensson, 1978; Nyhlen, 1979; Nyhlen and Unestam, 1980). The zoospores drop their flagella and encyst on the cuticle. The zoospore can encyst up to three times if the initial site is not found to be suitable (Cerenius and Söderhäll, 1984). The "cysts" are not a true resting stage; however, the survival time of these "cysts" is from 1 to 2 days (Söderhäll and Cerenius, 1987). Sites on the joints or between the abdominal segments are the most successful sites for germination of the encysted zoospore.

Germination proceeds with lipolytic enzymes from the zoospore penetrating the outer lipid layer of the cuticle (Svensson, 1978). A germination tube or penetration peg is formed which penetrates the cuticle (Svensson and Unestam, 1975). Once the germ tube has penetrated the cuticle, hyphae with chitinase and protease activities begin to develop (Söderhäll and Unestam, 1975; Hall and Söderhäll, 1983). The hyphae most often grow parallel to the chitin fibrils within the cuticle (Nyhlen and Unestam, 1975; Svensson, 1978).

### 5.1.3. Epizootiology

The oomycete fungus *Aphanomyces astaci* causes disease and mortality in many species of freshwater crayfish. This fungus has not been reported in Australia. The fungus is pathogenic to the European species of freshwater crayfish and has caused up to 100% mortality in the freshwater crayfish *A. astacus*, *A. leptodactylus*, and *Austropotamobius pallipes* in Europe. Australian species of freshwater crayfish are susceptible to the disease (Unestam, 1975; Roy, 1993) with mortalities of nine different crayfish species including yabbies (*C. destructor*), gilgie (*C. quinquecarinatus*) and red claw (*C. quadricarinatus*) being demonstrated under experimental conditions. The freshwater crab *Eriocheir sinensis* has also been experimentally infected with the fungus (Benisch, 1940).

*Aphanomyces astaci* is specific to freshwater crayfish surviving for only a relatively short time outside a crayfish host (Unestam, 1969a, 1972). There is no known intermediate or secondary host of the fungus. *Aphanomyces astaci* infects a range of crayfish particularly European species. North American crayfish species such as *Procambarus clarkii*, *Orconectes limosus*, and *Pacifastacus leniusculus* are more resistant to crayfish plague than other freshwater crayfish (Unestam, 1969a, 1975) and only succumb to fulminating infection under conditions of stress (Persson and Söderhäll, 1983; Vey et al., 1983; Diéguez-Urbeondo et al., 1993). In the North American species, the fungus and host appear to exist in a stable host-parasite relationship which is not the case with the fungus and the European species of freshwater crayfish (Svardson, 1992; Diéguez-Urbeondo et al., 1993). However, it should be noted that North American crayfish such as *Pacifastacus leniusculus*, *Orconectes limosus*, and *Procambarus clarkii* are carriers of the crayfish plague fungus and thus will function as vectors for this disease. They can transmit the disease to other crayfish in a water body. Although birds and other aquatic animals such as otter or mink have been considered as possible carriers of zoospores between watersheds, this has not been established (Nylund and Westman, 1992).

Death of the crayfish usually occurs 1–2 weeks after initial infection. However, this can take much longer, with periods of up to 5 weeks being recorded. The disease progresses more rapidly at higher temperatures (Unestam, 1972, 1975; Vey et al., 1983; Persson and Söderhäll, 1983).

Outbreaks of the disease have caused heavy mortalities of European crayfish species in Europe since 1859 (Cornalia, 1860). The disease has been reported in the United Kingdom, Ireland, France, Spain, Turkey, Germany, Greece, Norway, Sweden, Finland, Austria, Slovenia, and the former USSR (Alderman, 1996). Significant economic damage to the freshwater crayfish industries occurred in Turkey following an initial outbreak of disease in 1984 (Gydemo, 1992) and in Finland in the early 1900s (Westman, 1991). In the United Kingdom, the spread of the disease throughout the major southern river systems has led to concern for the adverse ecological and environmental impact of the loss of the native species of freshwater crayfish (Holdich and Rogers, 1992; Alderman, 1993; Foster and Slater, 1995).

It has long been suspected that *Aphanomyces astaci* was introduced into Europe with the importation of the North American species of freshwater crayfish which act as vectors or carriers of the disease (Vey et al., 1983; Cerenius et al., 1987; Diéguez-Urbeondo and Söderhäll, 1993). Recent random application of polymorphic DNA-PCR (RAPD-PCR)

studies of strains of *Aphanomyces astaci* have shown that it is highly probable that the fungus was introduced with crayfish from North America (Huang et al., 1994; Diéguez-Urbeondo et al., 1995; Oidtmann et al., 1999).

It is interesting to note that no disease has been recorded in recent times from Italy or Portugal, even though North American species are present in these countries together with susceptible European species of crayfish (Westman and Westman, 1992; Perez et al., 1997). Also of note is that while North American species of freshwater crayfish have been successfully imported into many different regions (Huner, 1994), including regions where susceptible European or Australian species of freshwater crayfish are also cultivated, crayfish plague outbreaks have not been reported. China is one such region. However, since few countries have official crayfish disease records or diagnostic and control measures in place (Westman and Westman, 1992), the lack of reports may not be indicative of the absence of crayfish plague outbreaks.

North American crayfish with melanized lesions containing *Aphanomyces astaci* may be responsible for maintaining a reservoir of infection by which susceptible species in the same watershed become infected (Persson and Söderhäll, 1983; Cerenius et al., 1987; Diéguez-Urbeondo and Söderhäll, 1993; Nylund and Westman, 1995). Successful long-term reintroduction of susceptible species of crayfish after an outbreak of crayfish plague in a body of water can occur only if all of the crayfish in the water source are completely eradicated before the reintroduction (Fürst, 1995). Collecting nets and equipment have been considered a major source of infection for populations of susceptible crayfish (Alderman, 1996) and are suspected of having caused the outbreaks of disease in Ireland (Holdich and Rogers, 1992). Boats, which have not been dried between watersheds and water containers being emptied in different watersheds are also considered to be responsible for spread of viable zoospores (Taugbol et al., 1993).

The spread of the disease is most rapid downstream during an outbreak (Taugbol et al., 1993; Nylund and Westman, 1995; Alderman, 1996). Spread upstream is much slower and takes place via the movement of crayfish. Barriers such as weirs and large tracts of water free of crayfish prevent further spread of the disease upstream (Taugbol et al., 1993).

Some methods that have been used in Norway (Taugbol et al., 1993) and Finland (Nylund and Westman, 1992) to control the spread of crayfish plague are

- Extensive publicity
- Crayfish must be boiled before being sold or moved from the place of capture.
- Collecting equipment from overseas must not be used.
- Collecting equipment is to be disinfected between seasons and between watersheds.
- Crayfish are not to be transferred between localities.
- Diseased or dead crayfish are not to be disposed of in watercourses.
- Boats and other items such as boots and equipment are to be completely dried when moved from one watershed to another.
- Water containers are not to be filled and emptied in different watercourses.
- Unfrozen fish are not to be taken to a different watercourse.

#### 5.1.4. Pathology

Gross signs of crayfish plague are variable, depending on the relationship between the severity of the infection from *Aphanomyces astaci* and the temperature at which infection occurs (Alderman and Polglase, 1988). If large numbers of zoospores are present, death is rapid and the animal exhibits few gross signs of disease other than a whitening of the tail muscle in severely affected areas. If fewer zoospores are present, the infection proceeds more slowly and is generally evidenced by brown melanization in the exoskeleton.

In the susceptible crayfish, there is initially an increase in activity, often during the day, followed by reduced motility (Nylund and Westman, 1992). Loss of limb coordination and paralysis occur in the later stages of the disease. A neurotoxin is suspected of contributing to these signs of disease (Unestam and Weiss, 1970).

Infection of host tissues by *Aphanomyces astaci* results in the release of phenolic compounds that are part of the host defense mechanism of crayfish (Söderhäll et al., 1979; Söderhäll and Ajaxon, 1982). These phenolic compounds are involved in the melanization reaction that occurs around the hyphae of *Aphanomyces astaci* within the cuticle. This reaction is most pronounced in resistant species of crayfish and often occurs around the growing tip of the hyphae thus inhibiting further invasion (Nyhlen and Unestam, 1980). In susceptible species, the melanization reaction is less distinct and is around the older part of the hyphae rather than the growing tip (Unestam and Söderhäll, 1977). Other immune mechanisms are also likely to be triggered by pathogen invasion. These have been extensively studied by Söderhäll (1981) and Söderhäll and Cerenius (1992).

Few lesions are seen in susceptible crayfish during the course of the disease (Jarvenpaa et al., 1986). After death, some mycelia may be seen (Vey, 1986). In resistant species of crayfish, such as *Pacifastacus leniusculus*, brown or black melanized spots containing hyphae may be present especially in the legs, abdomen, chelae, and mouth parts (Nylund and Westman, 1983, 1995).

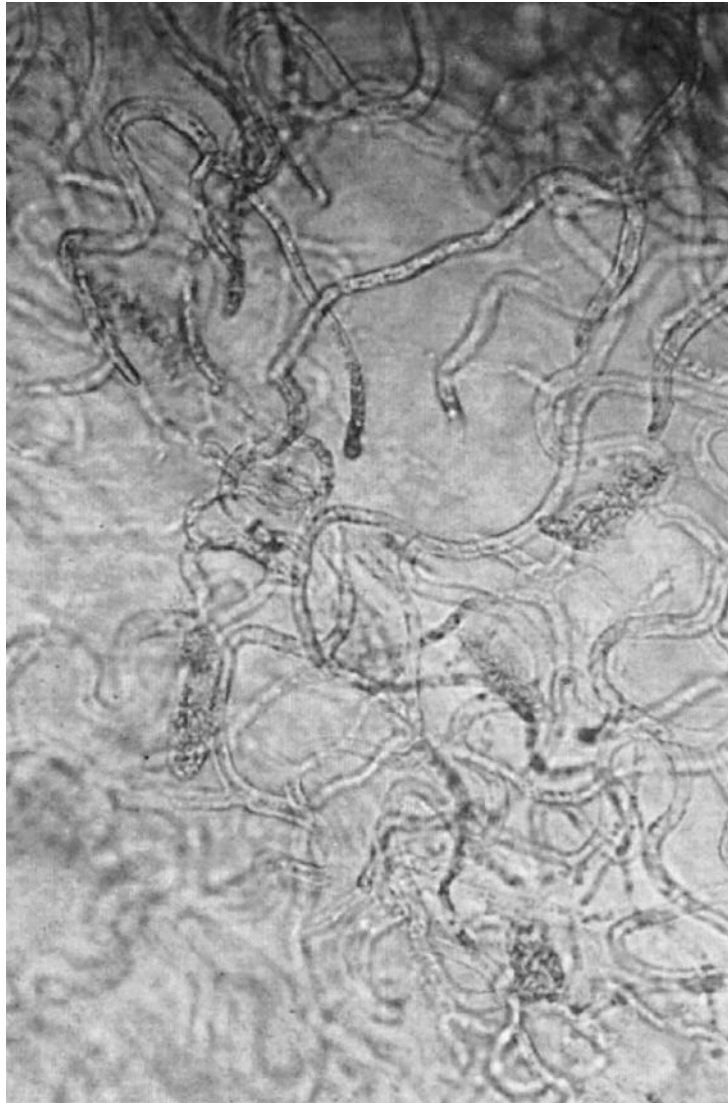
It is not easy to determine whether a crayfish is free of *Aphanomyces astaci* (Smith and Söderhäll, 1986; Cerenius and Söderhäll, 1992). Four main methods of detection and diagnosis are used

- History—Sudden deaths or disappearance of susceptible crayfish populations.
- Direct light microscopy of recently dead crayfish. Parts of the soft abdominal cuticle are examined for the typical nonseptate hyphae that are 7–10  $\mu\text{m}$  in diameter (Fig. 10). The hyphae have rounded tips. The mycelia grow within the cuticle.
- Culture and isolation of the fungal hyphae and zoosporangia. Bacterial and saprophytic fungal overgrowth is a problem when isolating *Aphanomyces astaci* (Cerenius et al., 1987). Cuticle to be studied is washed and placed on peptone-glucose agar together with antibiotics or 0.05% potassium tellurite inside a 1  $\times$  0.5 cm ring that has been partly embedded in the agar (Cerenius et al., 1987). After a few days at 20°C, the mycelia growing outside the ring are subcultured onto another medium for 2–3 days

after which time the cultures are examined for typical mycelium and zoosporangium morphology.

A method for isolating *Aphanomyces astaci* using RGY agar has been described (Alderman and Polglase, 1986). Antibiotics are sometimes used to control the growth of secondary invaders such as bacteria and saprophytic fungi (Alderman and Polglase, 1986; Taugbol et al., 1993).

- Transmission from an infected crayfish to susceptible crayfish under laboratory conditions.



**Figure 10.** *A. astacus* infected with *Aphanomyces astaci*. Fresh preparation of abdominal cuticle exhibiting three-dimensional hyphae pattern. (Photograph by Birgit Oidtmann, University of Munich.)

### 5.1.5. Pathogen viability

Zoospores remain viable in the mucus of unfrozen fish (Hall and Unestam, 1980; Nylund and Westman, 1995), and can survive for up to 2 weeks in mud (Rennerfelt, 1936). The survival time is strain and temperature dependent (Diéguez-Uribeondo et al., 1995).

Some conditions limit the growth of mycelia and spores of *Aphanomyces astaci*. Mycelia and spores do not survive drying for 48 h, freezing to  $-20^{\circ}\text{C}$  for 2 h and incubation temperatures of  $30^{\circ}\text{C}$  for 30 h (Smith and Söderhäll, 1986).

Sodium hypochlorite at 100 ppm free chlorine, and iodophores at 100 ppm iodine, are useful disinfectants (Alderman and Polglase, 1985). Organic matter was found to decrease the effectiveness of iodophores in the latter study. Malachite green (1.0 ppm) is an effective fungicide against *Aphanomyces astaci* (Hall and Unestam, 1980; Alderman and Polglase, 1984a).  $\text{MgCl}_2$  prevented the transmission of *Aphanomyces astaci* between crayfish by reducing sporulation (Rantamaki et al., 1992).

## 5.2. Condition: *Fusarium* infection (fungus disease)

### 5.2.1. Causative agent

*Fusarium* species including *Fusarium solani*, *F. oxysporum*, *F. tabacinum*, *F. roseum* var. *culmorum*.

Isolates of *F. solani* were obtained by surface disinfection of infected tissue with sodium hypochlorite and antibiotic solutions followed by incubation on malt agar at  $25^{\circ}\text{C}$  (Chinain and Vey, 1988). The colonies were white and later developed a rose pink color. *F. tabacinum* may be isolated on RGY agar with streptomycin sulphate and penicillum G incubated at  $16^{\circ}\text{C}$  (Alderman and Polglase, 1984b).

### 5.2.2. Epizootiology

Species of *Fusarium* are widespread and often are found in soil and plants. They have also been reported as infecting marine and freshwater Crustacea. In freshwater crayfish, the fungus is considered to be an opportunistic pathogen causing infection after stressors such as wounding or water pollution have decreased the resistance of the animal. Conditions that favor the infection of crayfish are common in aquaculture situations and high losses can be experienced (Maestracci and Vey, 1987).

*F. solani* can cause disease in the crayfish species *A. leptodactylus* and *Pacifastacus leniusculus* in Europe (Chinain and Vey 1987a,b, 1988). *A. leptodactylus* was the more susceptible species. Maestracci and Vey (1987) studied disease caused by *F. oxysporum* in the European species of freshwater crayfish *A. leptodactylus* and *Austropotambius pallipes*. *F. tabacinum* was reported as causing mortality in *Austropotambius pallipes* (Alderman and Polglase, 1984b). Other species of *Fusarium* such as *F. roseum* var. *culmorum* have been found to cause gill disease in freshwater crayfish (Vey, 1979). A *Fusarium* fungus has also been described in the North American crayfish, *Procambarus simulans simulans* but no histopathology was performed in that study (Lahser, 1975).

Death can occur up to several months after infection as the disease can be relatively slow to develop (Alderman and Polglase, 1984b; Chinain and Vey, 1987a,b, 1988). Death has

been attributed to physiological disturbances resulting from interference with molting, exotoxin production by the fungus and disturbances of osmotic pressure and sodium and chloride ion concentration in the haemolymph (Alderman and Polglase, 1985; Maestracci and Vey, 1987; Chinain and Vey, 1988).

The rate of mortality can vary between crayfish species, species of *Fusarium* and whether crayfish are injured or uninjured at the time of infection. Mortalities increased under some culture conditions and during or after a molt (Chinain and Vey, 1988). Secondary bacterial infection can also contribute to the death of the crayfish (Vey, 1986). Mortality under experimental conditions has been up to 100% (Maestracci and Vey, 1987).

Disease caused by *Fusarium* spp. have been reported in crustaceans in Australia, for example, *F. solani* in the western rock lobster (McAleer, 1983), but there appear to have been no reports of infection with *Fusarium* in Australian freshwater crayfish. There are many strains of *F. solani* that are pathogenic to marine crustaceans, plants and freshwater crayfish (Chinain and Vey, 1987a). Isolation and characterization of the different strains of *F. solani* is likely to be important in establishing the virulence to a particular host.

#### 5.2.3. Pathology

In some hosts, infection with *Fusarium* spp. results in lesions in the cuticle, gills, and haemocoel of freshwater crayfish. A melanization reaction occurs around the fungal hyphae leading to the presence of large brown patches in the cuticle and an intense cellular defense reaction in underlying tissues. The hemocyte aggregations in turn lead to formation of large encapsulations or granulomas. The diffuse melanization seen in this condition has led to the disease being described as "brown abdomen disease" (Chinain and Vey, 1988). In gills, brownish spots are present and there are widespread changes in the gill epithelium (Maestracci and Vey, 1987).

#### 5.2.4. Pathogen viability

Little is known of the viability of the organism in freshwater crayfish and freshwater crayfish products.

### 5.3. Condition: fungal infections and infestations (other than crayfish plague, *Fusarium* spp. infections, and shell disease)

#### 5.3.1. Causative agents

Fungi such as *Saprolegnia parasitica*, *Trichosporon beigeli*, *Achyla* sp., *Aphanomyces laevis* and trichomycete fungi.

#### 5.3.2. Epizootiology

Several saprophytic fungi can cause disease in freshwater crayfish that are wounded or stressed by water treatments or poor water quality (Alderman and Polglase, 1988; Gydemo, 1992). Unestam (1973) warned of the danger of mistaking saprophytic fungi for pathogenic species. Examples of saprophytic fungi that have caused lesions in freshwater crayfish are *S. parasitica* and *T. beigeli*. These fungi are pathogens of fish and mammals, respectively (Söderhäll et al., 1993; Diéguez-Urbeondo et al., 1994a,b). The finding of these fungi in

freshwater crayfish has raised questions about the potential for crayfish to act as vectors that may transmit the fungi to other susceptible species (Cerenius and Söderhäll, 1992; Söderhäll et al., 1993; Diéguez-Uribeondo et al., 1994a,b).

Two strains of *Aphanomyces laevis* have been demonstrated to infect wounds in *Procambarus clarkii*. Infectivity trials with these crayfish did not lead to fungal infections or mortalities, but it was suggested that mortality could occur in crowded, unhealthy conditions (Smith, 1940).

*S. parasitica* is an Oomycete fungus that is related to the freshwater crayfish pathogen *Aphanomyces astaci*. The fungus produces motile zoospores that are chemotactically attracted to wound exudates of freshwater crayfish, in much the same way as occurs with *Aphanomyces astaci* zoospores (Diéguez-Uribeondo et al., 1994a). Experimental infection of the crayfish species *A. astacus*, *A. leptodactylus*, and *Procambarus clarkii* revealed all three species to be equally susceptible to the disease (Diéguez-Uribeondo et al., 1994b). Mortality of up to 20% was noted in uninjured crayfish after exposure to zoospores. The mortality increased approximately threefold when injured crayfish were exposed. Deaths occurred over a period of several weeks, but the deaths occurred more rapidly in injured crayfish than in uninjured crayfish. As a result of these findings, it was concluded that *S. parasitica* could be carried as a chronic, benign infection in the cuticle of crayfish for several months. Death could result in a few of these crayfish under certain conditions either from the fungal infection or from invasion of the crayfish by other pathogens.

*T. beigelii* was found by Söderhäll et al. (1993) in the cuticle of *A. astacus* that had been stressed for a period of time in an experiment that was testing the effectiveness of MgCl<sub>2</sub> for preventing the transmission of *Aphanomyces astaci* between crayfish. Up to 50% of the crayfish died presumably due to immunocompromisation from the MgCl<sub>2</sub> exposure. The fungus growing in the cuticle had a similar growth pattern to that of *Aphanomyces astaci*.

Trichomycetes fungi are commonly found in the intestines and sometimes the cuticle of freshwater crayfish (Lichtwardt, 1962; Krucinska and Simon, 1968; Johnson, 1977). Trichomycetes are generally considered nonpathogenic in their arthropod hosts. *Achlya* sp., an Oomycete fungus, has been reported in the cuticle of freshwater crayfish in Europe (Krucinska and Simon, 1968; Vey, 1986) and Australia (Herbert, 1987).

Other species of fungi that have been reported as epizoots of freshwater crayfish include *Alternaria* sp., *Hormodendrum* sp., *Aspergillus* sp., *Saprolegnia* sp., *Uncinula* sp., and *Hormisum* sp. which were found on the external surfaces of one or more of the North American crayfish species *Procambarus simulans simulans*, *Procambarus clarkii*, *Procambarus zonalgulus*, and *Fallicambarus hedgpethi* (Lahser, 1975). In addition, a fungus belonging to Microecrinaceae has been reported to infest the gill cavity of *Cambarus affinis* and *A. leptodactylus* (Krucinska and Simon, 1968).

### 5.3.3. Pathology

Diagnosis of *S. parasitica* and *T. beigelii* is by the use of light microscopy of fresh preparations and culture using the same methods as for *Aphanomyces astaci* isolation (Cerenius et al., 1987). Other culture media recommended for isolation of saprolegniaceous fungi include corn meal agar and Sabouraud's agar (Bailey, 1994).



*S. parasitica* can cause melanized lesions in the cuticle of freshwater crayfish (Söderhäll et al., 1991; Diéguez-Uribeondo et al., 1994a,b).

A strong melanization reaction was noted in the cuticle of infected crayfish around the hyphae of *T. beigelii* (Söderhäll et al., 1993).

Trichomycetes fungi are commonly found in the intestines and sometimes the cuticle of freshwater crayfish (Lichtwardt, 1962; Krucinska and Simon, 1968; Johnson, 1977). Trichomycetes are generally considered nonpathogenic in their arthropod hosts. *Achlya* sp., an Oomycete fungus, has been reported in the cuticle of freshwater crayfish in France (Vey, 1986), on gill filaments of *Cambarus affinis* and *A. leptodactylus* (Krucinska and Simon, 1968) and infecting the gill filaments of moribund *C. quadricarinatus* from Queensland (Herbert, 1987). In the latter study, melanin deposition was observed around hyphae penetrating the gill filaments.

#### 5.3.4. Pathogen viability

There is little information available on the survival of *S. parasitica* zoospores or *T. beigelii* spores in the aquatic environment. Treatment of *Aphanomyces invadens* and other saprolegniaceous fungi with various antibiotics, fungicides, and disinfectants has been studied with limited success (Lilley and Inglis, 1997).

Trichomycete fungi produce two types of spores. One reinfects the gut of the host crayfish and the other passes out of the crayfish into the environment where it can infect other crayfish (Lichtwardt, 1962). Information on viability of spores under different treatment regimes is lacking but they have been described as being resistant to adverse environmental conditions (Lichtwardt, 1962).

### 5.4. Condition: fungal conditions of crayfish eggs

#### 5.4.1. Causative agents

*Saprolegnia* sp. and other oomycetes.

#### 5.4.2. Life cycle/life history

Primary zoospores encyst after swimming for a short period of time. The primary cysts form secondary spores that have two flagella and swim for a prolonged period. The secondary spore also encysts and then germinates after which hyphae are formed (Bailey, 1994).

#### 5.4.3. Epizootiology

Many Oomycetes are specialized parasites of egg masses or embryos and can infect the eggs of various species of crustaceans (Unestam, 1973). For example, *Aphanomyces ovidestruens* infects the eggs of freshwater copepods and causes a decrease in the population of these hosts (Gicklhorn, 1923; Burns, 1980).

*Saprolegnia* sp. invades dead eggs in the egg masses of freshwater crayfish. It then overgrows viable eggs in the same egg mass or infects an egg mass on another individual via zoospores (Vey, 1979, 1986). A Saprolegniales fungus was shown to infect eggs and moribund larvae of *C. quadricarinatus* (Herbert, 1987). Active, healthy larvae were not affected.

However, larvae that were damaged, temperature stressed or deformed were susceptible to infection by the fungus. In stage 2 larvae, all body tissues were invaded and consumed by the hyphae within 48 h at 25–27°C (Herbert, 1987).

#### 5.4.4. Pathology

The fungi invade egg masses and may result in 100% mortality.

#### 5.4.5. Pathogen viability

Treatment of infected crayfish eggs with malachite green at 0.1 ppm every 4 days has been shown to reduce infections and larval mortality.

### 5.5. Condition: shell disease (burn spot disease)

#### 5.5.1. Causative agents

Chitinolytic Gram-negative bacteria including *Pseudomonas* sp. and fungi such as *Ramularia* sp., *Didymaria cambari*, and *Fusarium* spp.

#### 5.5.2. Epizootiology

Shell disease is one of the most common and widespread crustacean diseases, affecting a range of species including lobsters (Fisher, 1988; Getchell, 1989), shrimp (Brock and Lightner, 1990), crabs (Sawyer, 1990), and freshwater crayfish. Crayfish species affected by the disease include *A. astacus*, *A. leptodactylus*, *Cambarus affinis*, and *Orconectes limosus* (Mann and Pipelow, 1938), *Austropotamobius pallipes* (O'Keefe and Reynolds, 1983), *Procambarus clarkii* (Amborski et al., 1975), *C. destructor albidus* (Mills, 1983; Evans et al., 1992), and *C. tenuimanus* (Owens and Evans, 1989; Evans et al., 1992).

The etiology of shell disease is not fully understood. Electron microscopic studies of early lesions has demonstrated a role for chitinolytic bacteria as initiating agents (Amborski et al., 1975). These bacteria must first gain entry through the epicuticle, either through superficial wounds or through microbial action. Chitinoclastic fungi are also found in the necrotic centers of the lesion and are thought to contribute to the development of the condition (Rosen, 1970). *Pseudomonas* sp. was the only chitinolytic bacterial species isolated from shell disease lesions in *Procambarus clarkii* but numerous other species including *Citrobacter freundii*, *Aeromonas liquefaciens*, *Pseudomonas alkaligenes*, *Enterobacter* sp., *Achromobacter* sp., and *Bacillus* sp. were also present, probably as secondary invaders (Amborski et al., 1975). Similar bacterial species were found in shell disease lesions in *C. tenuimanus* (Owens and Evans, 1989).

Fungal species reported as associated with shell disease lesions in freshwater crayfish include *Ramularia astaci* and *Didymaria cambari* (Mann and Pipelow, 1938). However, it has been suggested that the species identified as *R. astaci* in the early studies may have been *F. tabacinum* or a closely related species (Alderman and Polglase, 1984b).

Shell disease is usually seen in freshwater crayfish from dense populations (Unestam, 1973) or from populations being reared under suboptimal conditions (Thune, 1994). Metabolic disturbance or physical trauma, compounded by the activity of chitinoclastic microorganisms, are initiating factors (Sindermann, 1990). The incidence and severity of the

condition can increase when molting is infrequent, as occurs in nutritionally poor environments. In mild cases, the lesion is lost when the animal molts but deformities in the new shell occur, rendering the animal unsightly and reducing its market value. Mortalities associated with shell disease, particularly at the time of molting, have been reported for freshwater crayfish (Rosen, 1970; Unestam, 1973).

Shell disease in decapod crustaceans is reported to be contagious, and unaffected crayfish placed in the same water body as affected individuals are likely to acquire the disease (Rosen, 1970; Amborski et al., 1975). However, most efforts to transmit the disease, other than by placing an uninfected crayfish in contact with diseased ones, have been met with little success (Johnson, 1983). Numerous authors have emphasized the important role played by exoskeleton damage, crowding, and adverse environmental conditions as etiological factors (Rosen, 1970; Unestam, 1973; Fisher, 1988; Alderman and Polglase, 1988; Sindermann, 1990; Thune, 1994).

### 5.5.3. Pathology

Gross signs comprise brown colored lesions in the exoskeleton with depressed, necrotic centers. The appearance of the brown lesions led to the term "burn spot disease" to describe the condition. The lesions occur in the calcified parts of the exoskeleton, mostly on external surfaces including gill filaments. They commence as a small, darkened, sometimes friable or cratered area on the cuticle (Johnson, 1983). In freshwater crayfish, the ventral edge of the uropods is usually the first site of attack, but lesions can also occur in the cephalothorax, the abdomen, and the gills (Unestam, 1973; Amborski et al., 1975; Mills, 1983). In mild cases, the lesion is limited to the cuticular tissues, but in more severe cases the underlying soft tissue is involved. Severe erosion of the tail can occur (Amborski et al., 1975).

Light microscopic and histopathologic examination of lesions may show fungal hyphae and/or bacteria in the crayfish cuticle. Glucose-agar culture is used to isolate the fungus and to produce conidia (Unestam, 1973).

### 5.5.4. Pathogen viability

Studies on *Macrobrachium rosenbergii* have shown that a dip treatment with 10 ppm oxolinic acid is an effective treatment for burn spot lesions (El-Gamal et al., 1986). Infective agents in lesions should also be destroyed by exposure to high temperatures. Information on the effect of freezing on shell disease pathogens is lacking.

## 6. Protistan parasites

### 6.1. Condition: microsporidiosis; colloquially albino, cotton tail, or porcelain disease

#### 6.1.1. Causative agents

*Thelohania*-like spp., *Pleistophora*-like spp., *Ameson* sp., and *Vavraia parastacida*.

In general, microsporidian spores are acid fast and spores are generally ovoid in shape. Spore size varies between species, but a typical microsporidian spore is around  $5 \times 3 \mu\text{m}$ . *Thelohania*-like spp., *Pleistophora*-like spp., and *Vavraia parastacida* spores are packaged

within pansporoblasts. The number of spores within each pansporoblast is taxonomically important: *Thelohania*-like spp. have 8 spores, *Pleistophora*-like spp. have many more and numbers are variable, and *Vavraia parastacida* has 8, 16, 32, and rarely 64 spores per pansporoblast. *Ameson* spp. have single spores. Microsporidian spores typically consist of a coiled polar tube, a membranous polaroplast, and often a spongy posterior vacuole.

### 6.1.2. Life cycle/life history

The life cycle of crayfish microsporidians is poorly understood. However, present information concerning transmission suggests that life cycles may differ between related species. While speculative, the possibility exists that other arthropods are involved in the life cycle of some crayfish microsporidians, as some microsporidians utilize both copepods and insects as hosts (Sweeney et al., 1985; Andreadis, 1985).

### 6.1.3. Epizootiology

Microsporidians are intracellular parasites belonging to the protistan phylum Microspora. Microsporidians infect many vertebrates and invertebrates and are common in freshwater crayfish (Table 1). Microsporidiosis has been called the most significant disease of freshwater crayfish globally, aside from crayfish plague (Alderman and Polglase, 1988). The taxonomy of crayfish microsporidians is obscure; hence it is difficult to determine which strains or species have been described in separate regions. Consequently, *Thelohania contejeani* may not occur outside of Europe as the literature suggests.

**Table 1.** Reports of microsporidians in freshwater crayfish

Name used	Host species	Location	Reference
<i>Ameson</i> sp.	<i>Austropotamobius pallipes</i>	England	Pixell Goodrich, 1956
<i>Pleistophora</i> sp.	<i>Cambarellus puer</i>	Louisiana, USA	Soganders-Bernal, 1962
	<i>Cherax destructor</i>	South Australia	O'Donoghue et al., 1990
<i>Thelohania cambari</i>	<i>Cambarellus bartoni</i>	Georgia and North Carolina, USA	Soganders-Bernal, 1962
<i>Thelohania contejeani</i>	<i>Astacus astacus</i>	France	Henneguy and Thelohan, 1892; cited in Alderman and Polglase, 1988
	<i>Astacus astacus</i>	East Germany	Schaperclaus, 1954; cited in Alderman and Polglase, 1988
	<i>Astacus astacus</i>	Russia	Voronin, 1971
	<i>Astacus astacus</i>	Russia	Mazylyis, 1978
	<i>Astacus astacus</i>	Yugoslavia	Obradovic and Sabocenc, 1983
	<i>Astacus astacus</i>	Finland	Sumari and Westman, 1969
	<i>Astacus astacus</i>	Norway	Skurdal et al., 1988, 1990
	<i>Astacus leptodactylus</i>	Poland	Krucinska and Simon, 1968
	<i>Austropotamobius pallipes</i>	France	Vey and Vago, 1973
	<i>Austropotamobius pallipes</i>	Northumberland, UK	Brown and Bowler, 1977
<i>Cambarus affinis</i>	Poland	Krucinska and Simon, 1968	

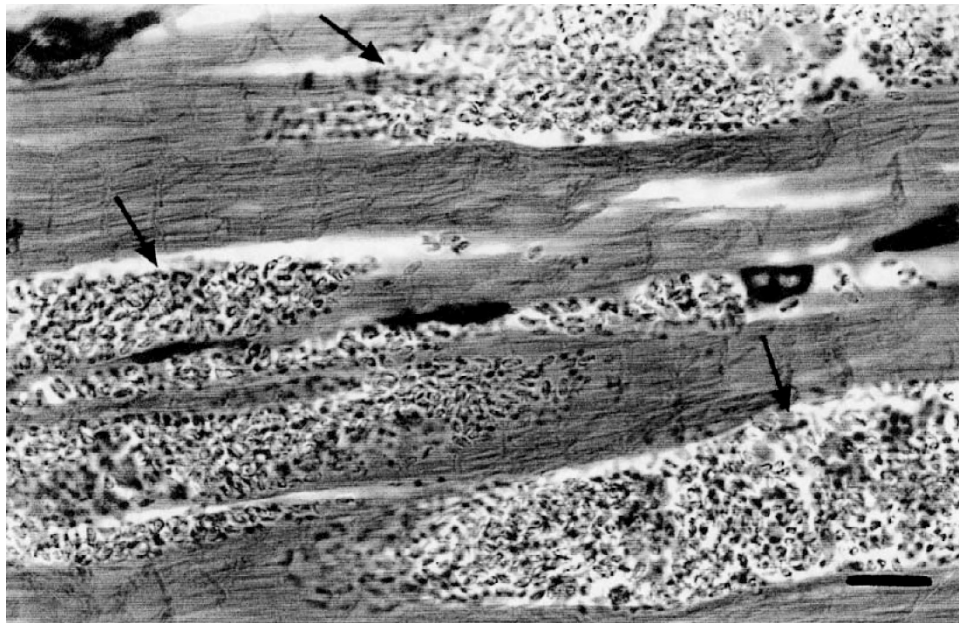
	<i>Orconectes virilis</i>	Ontario, Canada	France and Graham, 1985
	<i>Paranephrops zealandicus</i>	Dunedin, New Zealand	Quilter, 1976
<i>Thelohania soganderesi</i>	<i>Cambarellus shufeldti</i>	Louisiana, USA	Soganders-Bernal, 1962
<i>Thelohania</i> sp.	<i>Austropotamobius pallipes</i>	Ireland	Reynolds, 1978
	<i>Austropotamobius pallipes</i>	England	Pixell Goodrich, 1956
	<i>Cherax destructor</i>	Southern Australia	Carstairs, 1978
	<i>Cherax quadricarinatus</i>	Northern Australia	Herbert, 1988
	<i>Cherax destructor</i>	South Australia	O'Donoghue et al., 1990
	<i>Paranephrops planiformis</i>	Lake Taupo, New Zealand	Jones, 1980
<i>Vavraia parastacida</i>	<i>Cherax tenuimanus</i>	Western Australia, Queensland	Langdon, 1991a,b Langdon and Thorne, 1992 Evans et al., 1992
	<i>Cherax quadricarinatus</i>		
	<i>Cherax quinquecarinatus</i>		
	<i>Cherax destructor albidus</i>		

Direct transmission by cannibalism of infected crayfish appears to vary at least between microsporidian species. Graham and France (1986) and Herbert (1988) were unable to transmit *Thelohania*-like spp. directly to crayfish by feeding infected tissues. Also Oidtmann et al. (1997) and Hoffmann et al. (1999) report that they could not induce transmission of *Thelohania contejeani* to *A. astacus* by feeding or injecting spore-containing material. On the other hand, Overstreet (unpublished data) has successfully transmitted a *Thelohania*-like sp. via this route. *Vavraia parastacida* is transmissible to *C. tenuimanus* and *C. destructor albidus* by cannibalism (Langdon and Thorne, 1992). These authors reported clinical infections in *C. destructor albidus* within 4 months postinfection, while infections in *C. tenuimanus* were subclinical in the same period.

#### 6.1.4. Pathology

Gross signs of microsporidiosis are relatively consistent in crayfish, as with other crustaceans, and include lethargy and opacity of musculature systemically. In late-stage infections, the whole of the abdominal musculature is chalky white rather than translucent; consequently, affected crayfish are commonly called albinos, cotton tails, or porcelain crayfish. Early stages of infection typically present multifocal opacity of musculature.

Microsporidians are intracellular, principally myotropic, parasites (Fig. 11). Clusters of microsporidian spores, along with other parasite stages, occupy a specific area such as the center of muscle fibers, leaving the margins uninfected. Infected tissues are typically inflamed. *Thelohania*-like spp. spores are also observed in heart, gonad, connective tissues, neural tissues, and haemolymph (Voronin, 1971; Cossins, 1973; Vey and Vago, 1973; Cossins and Bowler, 1974). Sporophorous vesicles or spores of *Vavraia parastacida* are also observed in the hepatopancreas, eye, heart, antennal gland, gill, and connective tissues (Langdon, 1991a).



**Figure 11.** Thelohianiasis in *A. astacus*. Note large aggregations of microsporidians (arrows) in muscle bundles. Scale bar = 16  $\mu$ m. H&E.

#### 6.1.5. Pathogen viability

Little is known about the viability of these crayfish microsporans. Studying a crab species, *Ameson michaelis*, Overstreet noted that single doses of Buquinolate (Overstreet, 1975) and Monesin (Overstreet, 1988) would inhibit development. Overstreet and Whatley (1975) found that a 5-min bath in a dilute solution of either sodium hydrochlorite or an iodine-containing disinfectant was an effective control measure. The same authors found that freezing of spores at  $-22^{\circ}\text{C}$  for 67 days was not completely effective in killing spores.

### 6.2. Condition: infections with internal facultative ciliates

#### 6.2.1. Causative agents

Facultative ciliates such as *Tetrahymena pyriformis*.

The causative agents of this condition have to be identified on a case-by-case basis with attention to critical details. In the case of *Tetrahymena pyriformis*, the ciliate was  $52 \times 36 \mu\text{m}$  and had 20–26 longitudinal kineties arranged in meridional rows (Edgerton et al., 1996). All meridians, with the exception of two, reached the anterior pole or the suture above the buccal apparatus. The other two meridians terminated at the posterior border of the buccal apparatus, which consisted of a paroral membrane on the right and a tripartite adoral zone of membranelles on the left. The ciliate did not possess a caudal cilium, and the length of somatic ciliature was consistent. The ciliate contained one to two posteriorly placed contractile vacuole pores. The macronucleus was irregularly ovoid to elliptical in shape and was central, next to a single spherical micronucleus.

### 6.2.2. Epizootiology

Crustaceans, especially cultured ones, are naturally susceptible to a variety of ciliates, some of which may be free living (Overstreet, 1987). Free-living species thrive in the body fluids of many crustaceans including crayfish. In Australia, one study revealed heavy infections of a ciliate identified as *Tetrahymena pyriformis* in 3 of 32 moribund *C. quadricarinatus* collected for histopathological examination during a 6-month study of chronic low-grade mortalities at a culture facility in North Queensland (Edgerton et al., 1995). A second study (Edgerton, 1996) failed to reveal such infections. Nevertheless, whether in Australia or abroad and whether in crayfish or other crustaceans, infections with free-living species occur and can affect commercial production of crustaceans.

Free-living ciliates found in endoparasitic situations can be considered facultative parasites. Under most conditions, the same ciliates live among or in association with the crayfish without invading or causing infections. However, if the resistance of the crayfish is reduced for any of several reasons, and a wound in the integument allows a threshold number of an agent to enter a host, then the host cannot prevent rapid reproduction of that agent. Infections are typically associated with young crustaceans and poor water quality. When animals are healthy, spread of the abundant agent can be halted. However, dying animals being fed on by other stressed cohorts under some situations can transmit the condition to others. Once a normal healthy crayfish dies, ciliates invade the tissues, feed and rapidly reproduce. This is one reason why dead crayfish should not be mixed with living animals ready for market.

### 6.2.3. Pathology

In the example case above, individuals of *C. quadricarinatus* infected with *Tetrahymena pyriformis* were lethargic, had a weakened or failed tail-flick response, and were unable to right themselves when placed on their back (Edgerton, 1996; Edgerton et al., 1996). Ciliates were visible in the haemocoel of the gills in wet mounts. Subclinical infections were not detected. This should be a good representation of clinical signs expected from a heavy infection in any crustacean with any free-living ciliate. Histologically, the haemocoel in the gills of the Australian example was occluded with *Tetrahymena pyriformis*, which infiltrated all organs (Fig. 12), causing extensive necrosis, particularly in the hepatopancreas and antennal gland. Lipid reserves in the hepatopancreas were often high in infected individuals, suggesting a rapid pathogenesis following infection.



**Figure 12.** *Tetrahymena pyriformis* (arrows) in between hepatopancreatic tubules in *C. quadricarinatus*. Scale bar = 32  $\mu$ m. H&E.

#### 6.2.4. Pathogen viability

Little is known about the viability of ciliates in crustacean products, but *Tetrahymena pyriformis* did survive storage in *C. quadricarinatus* tissues overnight at 4°C (Edgerton, unpublished data).

### 6.3. Condition: infections or infestations with apostome ciliates

#### 6.3.1. Causative agents

Apostome ciliates.

Apostomes have sparse somatic and oral ciliature (Morado and Small, 1995). Somatic ciliature is sometimes in rows. Oral structures often form a rosette consisting of infolded membranes with single cilia or kinetosomes at their bases. The cytostome is internal and is connected to the surface by a tubelike structure. Some apostomes have a thigmatactic field.

#### 6.3.2. Epizootiology

Some apostome ciliates infect the gills of freshwater crayfish, both internally and externally (Grimes, 1976; Johnson, 1977; Overstreet, unpublished data). External apostomes encyst on the gill surface and are nonpathogenic. Most apostomes infesting freshwater crayfish are in this category. However, some species perforate the gill cuticle, invade underlying gill tissues, and are pathogenic. Common genera in North American fresh and brackish waters are *Hyalophysa*, *Gymnodinioides*, and *Terebrospira* (Johnson, 1977). Apostome ciliates have not been reported from Australian crayfish.



Trophonts of external apostomes hatch from their cysts once the crayfish molts, feeding on fluids trapped within the exuvium. Mature trophonts encyst on an appropriate substrate and divide to form tomonts. The motile tomonts then locate a suitable host and encyst. The degree of host specificity depends on the particular apostome species; *Hyalophysa lwoffi*, an apostome species infesting freshwater crayfish, has also been found on several freshwater prawn species (Bradbury and Clamp, 1973; Grimes, 1976).

#### 6.3.3. Pathology

Internal apostome ciliates cause necrosis of tissues and produce a haemocytic response with pigmentation which produces a black speckled appearance of the gills (Overstreet, 1978). Apostome ciliates encysted on gills and general body exoskeleton may cause focal inflammation and melanization at the site of attachment or around it.

#### 6.3.4. Pathogen viability

Nothing is known about the viability of apostome cysts subjected to various physicochemical conditions.

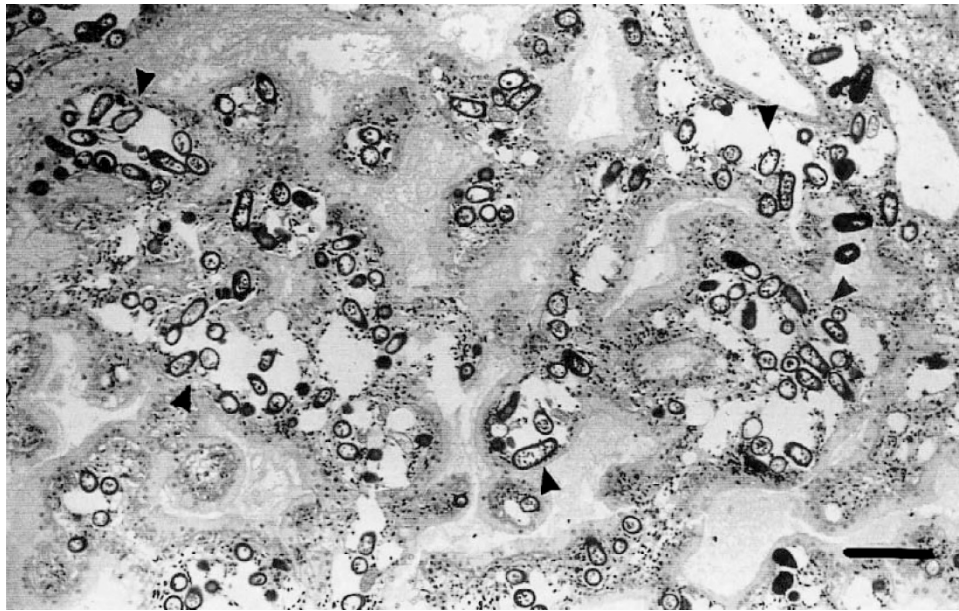
### 6.4. Condition: *Psorospermium* infection

#### 6.4.1. Causative agents

*Psorospermium haeckeli* and similar *Psorospermium* spp.

Although originally described as a protozoan or sporozoan (Unestam, 1975; Vey, 1979; Lee et al., 1985), a nematode (Ljungberg and Monne, 1968) and a dimorphic fungus (Nylund et al., 1983; Herbert, 1987; Alderman and Polglase, 1988), a recent study has proposed a taxonomic affiliation of *Psorospermium haeckeli* and similar species with eukaryotic protists (Ragan et al., 1996). At least four different morphotypes of *Psorospermium* organisms can be distinguished based on size, morphology, and the method of embedding into the connective tissues of their host—two in Europe, one in the United States, and one in Australia (Vogt et al., 1996). Whether these are different strains of the same species or different species has yet to be determined.

The mature cyst form comprises an ovoid structure approximately 45–60 × 90–100 μm in size. It has a thick, multilayered wall and many highly refringent globules of varying sizes in the cytoplasm. The outermost layer is thin and amorphous and does not stain readily (Henttonen, 1996). The next layer is composed of irregular thick plates that are strongly eosinophilic when stained with H&E. An Australian form differs from those described from Europe and the United States in having two plates that join at the center of the spore to create a central, circular ridge (Fig. 13) (Edgerton, 1996; Evans and Jussila, 1997).



**Figure 13.** Heavy *Psorospermium* sp. infection in the antennal gland in *C. quadricarinatus*. Note that *Psorospermium* sp. (arrowheads) are predominantly in the haemal space surrounding the nephridial canal. Scale bar = 200  $\mu$ m. H&E.

#### 6.4.2. Life cycle/life history

The life cycle for *Psorospermium haeckeli* and similar species has not been fully elucidated but studies suggest a diphasic life cycle (Vogt and Rug, 1999). Several different life stages of the *Psorospermium* parasite have been described (Grabda, 1934; Rug and Vogt, 1994, 1995; Henttonen, 1996; Evans and Jussila, 1997) comprising early "amoeboid" forms and a resting "cyst" or "spore" form. No intermediate host has been identified.

#### 6.4.3. Epizootiology

*Psorospermium haeckeli* was first described as a parasite of freshwater crayfish by Haeckel (1857) and the same or similar organism(s) has since been observed in a range of crayfish species including *A. astacus* [Haeckel, 1857; Grabda, 1934 (called *Potamobius fluviatilis*); Ljungberg and Monne, 1968; Unestam, 1973a; Nylund and Westman, 1979; Fürst and Söderhäll, 1987; Henttonen et al., 1990; Cerenius and Söderhäll, 1992a], *A. leptodactylus* (Haeckel, 1857; Krucinska and Simon, 1968; Alderman and Polglase, 1988), *Austropotamobius torrentium* (Vey, 1979; Vranckx and Durliat, 1981; Vogt et al., 1996), *Pacifasticus leniusculus* (Unestam, 1973b; Vey, 1979; Diéguez-Uribeondo et al., 1993), *Cambarus affinis* (Krucinska and Simon, 1968), *Procambarus clarkii* (Lee et al., 1985; Henttonen et al., 1992), *Procambarus zonangulus* (Henttonen et al., 1992), *Orconectes limosus* (Scheer, 1979), *C. quadricarinatus* (Herbert, 1987; Edgerton et al., 1995), *C. quinquicarinatus* (Owens and Evans, 1989) and *C. tenuimanus* (Evans, 1988; Owens and Evans, 1989; Aiken, 1989; Evans et al., 1992; Henttonen, 1996; Evans and Jussila, 1997).

Variations in infection prevalence between different crayfish populations are common (see review, Henttonen, 1996) and probably reflect differences in environmental conditions, seasonal effects or species difference in host resistance to infection. The earliest age of infected *A. astacus*, *Procambarus clarkia*, and *Procambarus zonangulus* was about 2–4 months (Henttonen, 1996) and of *C. tenuimanus*, approximately 12 months (Evans and Jussila, 1997).

The transmission of the parasite is also unknown but infection through ingestion has been suggested (Grabda, 1934; Henttonen, 1996). Laboratory experiments in which crayfish of two different species, *A. astacus* and *Pacifastacus leniusculus*, were fed to each other in all combinations, has provided evidence that the signal crayfish, *Pacifastacus leniusculus*, can act as a vector for *Psorospermium haeckeli* to the noble crayfish, *A. astacus* (Gydemo, 1996).

#### 6.4.4. Pathology

*Psorospermium haeckeli* in astacid crayfish is found in highest concentration in connective and epidermal tissues under the carapace (Henttonen et al., 1994; Rug and Vogt, 1995; Vogt and Rug, 1995). In cambarid crayfish, on the other hand, very few of these parasites are detected in connective and epidermal tissues, the highest concentration being found in abdominal muscles (Henttonen et al., 1994). Studies with parastacid crayfish have shown a parasite distribution similar to that reported for astacid crayfish (Herbert, 1987; Edgerton et al., 1995; Evans and Jussila, 1997).

The pathogenicity of *Psorospermium* parasites to host organisms is uncertain. Some workers have reported a lack of hemocyte reaction to the organism and taken this to imply little or no pathogenic effect (Unestam, 1973a; Herbert, 1987). In contrast, a number of other studies have demonstrated hemocyte aggregations around encysted parasites (Ljungberg and Monne, 1968; Vranckx and Durliat, 1981; Rug and Vogt, 1995; Evans and Jussila, 1997), melanin deposition (Henttonen et al., 1995; Rug and Vogt, 1995), degranulation of semi-granulated hemocytes (Thörnqvist and Söderhäll, 1993) and activation of the prophenoloxidase activating system (Cerenius et al., 1991), all of which are recognized as host defense responses to injurious stimuli. Furthermore, crayfish infected with *Psorospermium* have been shown to have an increased susceptibility to infection by *Aphanomyces astaci* (Fürst and Söderhäll, 1987; Cerenius and Söderhäll, 1992b; Thörnqvist and Söderhäll, 1993). The presence of high parasite loads could influence susceptibility to other pathogens or disease-causing agents.

#### 6.4.5. Pathogen viability

Information on viability of *Psorospermium* is lacking. One study conducted with *A. astacus* naturally infected with *Psorospermium haeckeli* in which the crayfish were treated with 10 mg/ml of the triazine derivative, HOE 092 V, provided results suggestive of a detrimental effect on the parasite but definitive evidence of parasite mortality was lacking (Schmahl et al., 1992).

## 7. Metazoan parasites

### 7.1. Condition: digenean infections

#### 7.1.1. Causative agents

Digeneans belonging to several different families.

A variety of digeneans belonging to at least 10 families utilize freshwater crayfish as second intermediate hosts (Table 2). Because there is a wide range of members in diverse digenean family groups, the individual species, all hermaphroditic, have widely differing characteristics. Those of potential public health concern include *Paragonimus* spp. (at least three species). The metacercariae are relatively small (about 0.4 mm in diameter). Most other metacercariae are spherical to elliptical and roughly 0.2–0.3 mm in diameter. Excysted worms are either elongated or oblong, depending on the species. They typically have an anterior oral sucker and another sucker on the ventral surface. Specific identification of all species requires the original descriptions.

**Table 2.** Representative records of digeneans found in freshwater crayfish

Species	Host	Geographic range	Other hosts	Tropism	Reference
<i>Allocorrigia filiformis</i>	<i>Procambarus clarkia</i>	Louisiana, USA		AG	Turner and Corkum, 1977
<i>Alloglossoides caridicola</i>	<i>Procambarus zonangulus</i>	Louisiana, USA		AG	Corkum and Turner, 1977
<i>Alloglossoides dolandi</i>	<i>Procambarus epicyrtus</i>	Georgia, USA		AG	Turner and McKeever, 1993
<i>Alloglossidium progeneticum</i>	<i>Procambarus spiculifer</i>	Georgia, USA	<i>Ictalurus nebulosus</i> (catfish)	AG	Font and Corkum, 1975
<i>Alloglossidium greeri</i>	<i>Cambarus shufeldti</i>	Louisiana, USA		AG	Font, 1994
<i>Alloglossidium corti</i>	Species not given	Louisiana, USA	Catfish and <i>Macrobella ditetra</i> (leech)		Font and Corkum, 1977
<i>Alloglossidium renale</i>	Species not given	Louisiana, USA	<i>Palaemonetes kadiakensis</i> (freshwater prawn)		Font and Corkum, 1977
Cathaemasiidae sp.	<i>Cherax tenuimanus</i>	Western Australia		HP	Evans et al., 1992
<i>Crepidostomum cornutum</i>	Cambarid spp.	Louisiana, USA	Catfish, <i>Musculium</i> sp. (clam)	HP, He, M and Go	Soganders-Bernal, 1965
<i>Gorgodera amplicava</i>	<i>Orconectes palmeri creolanus</i> and <i>Procambarus clarkia</i>	Louisiana, USA	Amphibians	S	Soganders-Bernal, 1965
<i>Macroderoides typicus</i>	<i>Procambarus blandingi acutus</i> , <i>Procambarus clarkia</i> , and <i>Orconectes lancifer</i>	Louisiana, USA	Tadpoles	C	Soganders-Bernal, 1965
<i>Macroderoides progeneticus</i>	<i>Procambarus spiculifer</i>	Georgia, USA		AG	Sullivan and Heard, 1969

<i>Macroorchis spinulosus</i>	<i>Cambaroides similis</i>	Korea	Mice, rats, and cats	M	Chai et al., 1996
<i>Maritrema obstipum</i>	<i>Cambarellus shufeldti</i> and <i>Procambarus clarkia</i>	Louisiana, USA		Gi and HP	Soganders-Bernal, 1965
<i>Maritreminoides medium</i>	<i>Orconectes virilis</i> and <i>Orconectes propinquus</i>	USA		Gi	Alderman and Polglase, 1988
Microphallidae sp.	<i>Cherax tenuimanus</i>	Western Australia		M	Evans et al., 1992
<i>Microphallus opacus</i>	<i>Cambarellus puer</i> , <i>Orconectes propinquus</i> , and <i>Procambarus clarkia</i>	Louisiana, USA		HP	Soganders-Bernal, 1965; Caveny and Etges, 1971
<i>Microphallus minutus</i>	<i>Cherax dispar</i> , <i>Cherax destructor</i>	Australia	Water rats, mice, and chicks	M	Shimazu and Pearson, 1991; O'Donoghue et al., 1990
<i>Microphallus fonti</i>	<i>Procambarus clarkia</i>	Louisiana, USA		HP	Overstreet et al., 1992
<i>Ochetosoma</i> sp.	<i>Procambarus clarkia</i>	Louisiana, USA		M	Soganders-Bernal, 1965
<i>Opecoelus variabilis</i>	<i>Cherax depressus</i> , <i>Cherax dispar</i>	Queensland, Australia	Other freshwater decapods and many freshwater fish		Cribb, 1985
<i>Orchipedium isostomata</i>	All European crayfish spp.	Europe		HP	Alderman and Polglase, 1988
<i>Paragonimus kellicotti</i>	<i>Orconectes</i> spp., <i>Cambarus</i> spp., <i>Procambarus clarkia</i>	USA	<i>Pomatiopsis lapidaria</i> (snail) and mammals including humans	He	Soganders-Bernal, 1965; Soganders-Bernal and Seed, 1973; Overstreet, 1978; Beaver et al., 1984
<i>Paragonimus westermani</i>	<i>Cambaroides</i> spp., <i>Cambaroides similis</i> , <i>Procambarus</i> sp.	South East Asia	Pleurocerid and thiarid snails, various crustaceans, and mammals including humans	Gi, HP, He, Go, and M	Yokogawa, 1964; Fan et al., 1990
Plagiorchidae sp.	<i>Cherax tenuimanus</i>	Western Australia	tortoises	M	Evans et al., 1992
<i>Sogandaritrema progeneticus</i>	<i>Cambarellus puer</i> , <i>Cambarellus shufeldti</i> , and <i>Procambarus clarkia</i>	Louisiana, USA	<i>Ammicola peracuta</i> (snail)	C	Soganders-Bernal, 1965

AG—antennal gland, C—cephalothorax, Gi—gill, Go—gonad, He—heart, HP—hepatopancreas, M—muscle, S—stomach

### 7.1.2. Life cycle/life history

The typical digenean life cycle consists of a molluscan first intermediate host and a vertebrate definitive host, as well as a second intermediate host, which can be a crayfish. Some of those species infecting crayfish are generalists, and infect other invertebrates as second intermediate hosts, and some macroderoidids are unusual in that they develop to maturity in the crayfish. Some of those do not use a vertebrate final host, others use both a crayfish and a fish, and still others mature in a leech (Carney and Brooks, 1991). In most cases, the snail, the first intermediate host, is highly specific, placing a barrier to introduction, although some specific exceptions such as *Gorgoderia amplicava* presumably can have a variety of

clam, crayfish and frog hosts. In most cases, however, the crayfish host is highly specific. The definitive host for some microphallids can include any of a variety of birds, mammals and fishes. The species of *Paragonimus* can infect several different mammalian species.

#### 7.1.3. *Epizootiology*

As described above, many digeneans have complex life cycles. Others, such as several species of macroderoidids (e.g., species of *Alloglossidium*), mature in the antennal gland of crayfish.

#### 7.1.4. *Pathology*

Most of these digeneans usually do not cause disease in freshwater crayfish unless they are present in very high numbers or impair the functioning of vital organs. The macroderoidids include egg-producing species in the antennal gland. *Paragonimus* spp. (at least three species) are typically very small and are usually found in the pericardial or abdominal muscle areas, while adults reach quite large sizes encapsulating in the mammalian lungs (8–15 mm long). Most other metacercariae including some microphallids that may also have public health significance are found in the muscle or associated with the viscera. Most digenean metacercariae have two to five difficult-to-differentiate walls (of both parasite and host origin) that are clear in fresh material, although some have a thin cyst or encapsulation. In some cases, a dark pigmented host response is associated with deposited eggs (Turner, 1984).

#### 7.1.5. *Pathogen viability*

Encysted metacercariae of most species typically remain viable in host tissue for about a week, and even longer if kept cool. For example, Fan et al. (1990) found that the survival rate of *Paragonimus westermani* decreased over time, but a few individuals were still infective to vertebrates after 560 days when stored at 5°C. Furthermore, those authors accidentally froze the material twice during that period.

Toltrazuril (Baycox) used in doses of 1–50 µg/ml, even for 24 h, was not effective in treating encysted microphallid metacercariae (Caughey, 1991). Other antiparasitic drugs are available, but proper and specific usage has not been determined.

## 7.2. *Condition: cestode infections*

### 7.2.1. *Causative agents*

Tapeworm metacestodes (the stage infective to the vertebrate final host) that infect crayfish belong to at least the Hymenolepidae and the order Amphilinidea.

Cyclophyllidean metacestodes are typically small, spherical to elliptical, encapsulated cercoids with an invaginated scolex that may or may not possess a rostellum with diagnostic hooks. The metacestode, as well as the adult, has no alimentary tract but has an abundance of small calcareous corpuscles that can be viewed in squash preparations of fresh material when looking for rostellar hooks or when examining histological sections of tissue that had been fixed in buffered formalin. The calcareous corpuscles dissolve in AFA, Davidson's solution, or other fixatives containing acetic acid as typically used for crayfish

when sectioning specimens to examine for viral infections. The metacestode of *Vampirolepsis diminuta* is a 1-mm-diameter, cream-colored, rather amorphous structure. When in the host, it has a withdrawn scolex with four unarmed suckers, a retractable rostellum with a single circle of hooks, and a prominent cercomer, a structure containing the hooks remaining from the prior larval stage (O'Donoghue et al., 1990).

For the amphilinid *Austramphilina elongata*, the metacestode in *C. destructor* as reported by Rohde and Watson (1989) is up to 7 mm long and more developed than the cyclophyllidean. There are five pairs of hooks: one "normal" median, two submedian halberd shaped, and two lateral serrate ones. Frontal glands open into a proboscis anteriorly, and developing reproductive organs and ducts are present.

### 7.2.2. Life cycle/life history

Important for risk assessment is the potential for introduction of any of a variety of avian cyclophyllidean cestodes that may use crayfish as intermediate or as paratenic hosts. For example, Linstow (1889) provided anecdotal data on cysticercooids of *Hymenolepis collaris* and *H. tenuirostris* from the body cavity of European crayfish. Most avian cyclophyllideans use a rather specific arthropod or crustacean intermediate host and a specific avian host. However, some species that infect ducks and other birds common to aquaculture and man-made ponds may also infect a wider range of intermediate hosts, some possibly being transferred to crustacean paratenic hosts. Whereas it may be likely to introduce exotic infections to Australian crayfish by introducing the agents with noncrayfish hosts, one also could expect the alternative. Importing infected crayfish could allow for introduction of an infection to native crayfish or other arthropod/crustacean hosts, especially if the species had a wide specificity of avian hosts including common ducks or other birds. If the identification of *Vampirolepsis diminuta* is correct and it develops to maturity in the water rat as reported, it possibly requires a rather specific definitive host. Attempts to experimentally transmit the metacestode from Australian crayfish to laboratory rats were unsuccessful (O'Donoghue et al., 1990).

*Austramphilina elongata*, a parasite of Australian turtles, uses *C. destructor* as well as other freshwater crustaceans as the intermediate host (Rohde and Georgi, 1983). *Austramphilina elongata* uses the freshwater turtle *Chelodina longicollis* as its final host. Its infective larva penetrates freshwater crustacea and develops into a metacestode infective to the turtle. Few species exist in this rather unusual group of "cestodes," a group considered separate from the cestodes in most texts. Consequently, the role of crayfish in the life cycle of other species is not known.

### 7.2.3. Epizootiology

How common and specific the hymenolepids are is unknown. At least one species of hymenolepid and the only amphilinid reported from a crayfish occur in Australia. In South Australia, O'Donoghue et al. (1990) reported what could be the hymenolepid *Vampirolepsis diminuta* in 0.4% of 1948 wild specimens of *C. destructor*. None of the 600 farmed *C. destructor* examined in that study was infected. Four of 51 wild specimens of *C. destructor* were infected by *Austramphilina elongata*, and 15 of 22 specimens experimentally exposed to the larva revealed an infection after 90 days.

#### 7.2.4. Pathology

Cestodes are not pathogenic to freshwater crayfish. *Vampirolepsis diminuta* infects the intestinal mucosa of the crayfish. *Austramphilina elongata* infections occur in the abdominal muscle near the anus of the crayfish (Rohde and Georgi, 1983).

#### 7.2.5. Pathogen viability

As with juveniles of other helminths, the metacestode stage in the crayfish intermediate host probably can survive several days in refrigerated products. A period of freezing, however, kills the agent.

### 7.3. Condition: nematode infections and infestations

#### 7.3.1. Causative agents

Nematodes have separate sexes and an alimentary tract. Different species have different lips and cuticular structures, but they all have an anterior oral opening, an elongated muscular esophagus, an intestine, and an anus. The "free-living" kinds have an external ring of seta and a pair of well-developed amphids (sensory receptors). A good description of *Monhystera cambari* was presented by Chitwood (1935). The internal nematodes are strictly juveniles, so the adult diagnostic features have not developed, leaving identification to fine aspects of the alimentary tract, sensory papillae, and other cuticular structures. These roundworms do not have the setae or well-developed amphids of the external nematodes.

#### 7.3.2. Life cycle/life history

Some of the external "free-living" nematodes may require crayfish or other crustacean hosts to complete their life cycles. Details for these have not been elucidated. Prevalence and intensity of infestation seems to be at least partially determined by amount of organic material present and by temperature. Thus, their viability is probably dependent on both natural and processing conditions.

Internal species probably require a vertebrate definitive host to complete their life cycles. For example, spiruroids generally have an arthropod or crustacean intermediate host plus a bird or occasionally another vertebrate as a definitive host. *G. spinigerum* occurs in tumors in the stomach wall of a feline or dog definitive host. Its eggs extrude from those lesions and are ingested by and develop in a cyclopoid copepod. The juvenile develops in fishes, frogs, or snakes that eat the copepods, and it can be passed on to a variety of paratenic hosts such as crayfish until consumed by a definitive host.

#### 7.3.3. Epizootiology

Nematodes from crayfish both worldwide and in Australia have been poorly studied, but at least a few species occur in Australian hosts. In Australia, unidentified external species occur in farmed and wild crayfish from Western Australia (Evans et al., 1992), South Australia (Mills, 1983, 1986; O'Donoghue et al., 1990; Caughey, 1991), and Queensland (Low, 1995).

O'Donoghue et al. (1990) reported an unidentified spiruroid nematode that encapsulated in the intestinal wall of 1.8% ( $n = 1948$ ) wild and 0.8% ( $n = 600$ ) farmed *C. destructor*



in South Australia. An unidentified nematode was also observed encapsulated in striated muscle of the cephalothorax of *C. tenuimanus* (Evans et al., 1992).

As examples of some of these “free-living” infestations of crayfish gill chambers, Schneider (1932) reported 14 species in 8 genera (e.g., *Actinolaimus*, *Prochromadorella*, *Chromadorita*, *Dorylaimus*, *Monhystera*, *Rhabditis*, and *Trilobus*) in some crayfish (*Potamobius* sp. and *Cambarus* sp.) from Germany. Of all the nematode species, only *Prochromadorella astacicola* consistently was found associated with the gill chambers of crayfish. Another such species, *Monhystera cambari*, was reported from North Carolina, USA (Allen, 1933) and Mexico (Rioja, 1943). In South Australia, the monhysterid *Gammarinema* sp. infested all the specimens of *C. destructor* examined from the River Broughton and River Murray (Caughy, 1991). What appears to be the same species in Queensland infested from 0% to 94% of the farm-reared *C. quadricarinatus* throughout the state (Low, 1995).

Internal infections are not as readily observed as external ones, but they occur as exemplified by encapsulated *G. spinigerum* that apparently uses crayfish as paratenic hosts in Japan (Miyazaki, 1954).

Many nematode species not specific to crayfish or not reported from them in the wild could potentially infect crayfish, if appropriately exposed. For example, *Cambarus* sp. served as one of several paratenic hosts when it was experimentally infected with *Angiostromylus cantonensis*, the rat lungworm, by feeding it the infected intermediate snail host *Limnaea palustris* (Rachford, 1975). The reason for mentioning this example is that the nematode, already present in some areas of Australia, can infect a human as an accidental host, causing eosinophilic meningitis. For that matter, *G. spinigerum* is of public health concern; it can cause larval migrans in cutaneous and subcutaneous tissues or it can cause abscesses in the alimentary tract in humans, which are also probably paratenic hosts for the nematode (Beaver et al., 1984).

#### 7.3.4. Pathology

Nematodes associated with freshwater crayfish are located both externally and internally. Those species infecting or infesting (a term designating that the agent is involved with an external as opposed to an internal association) crayfish worldwide are poorly studied relative to their presence and their host-parasite associations as indicated above. The relationship between several nematode species reported from the gill surface of a crayfish and its host is not understood. The gill-dwelling species are in most cases free living. The fact that adult as well as juvenile specimens of a few species commonly infest a high prevalence of crayfish hosts and the lack of reports of these from free-living habitats indicates that there exists a close relationship between the nematode and its hosts. More than one species may infest a host, but the nematode groups involved are often involved with crustacean hosts. Because of the apparent relationship between a few external nematodes and their crayfish hosts, introduction of exotic “free-living” species may be more harmful to naive crayfish or other crustaceans.

#### 7.3.5. Pathogen viability

The viability of most nematodes is similar. For example, the paratenic species *Angiostromylus cantonensis* can remain infective in the snail carcass for up to 7 days (Richards and

Merritt, 1967). Alicata (1967) found that when the juvenile was exposed to 0°C for 24 h, it was still infective to a rat; however, if it was exposed to -15°C for 12 h, the worm completely lost its infectivity. Alicata (1967) also determined the thermal death point of *Angiostromylus cantonensis* to be between 50 and 55°C. Caughey (1991) exposed *Gammarinema* sp. to five doses of toltrazuril (Baycox, in doses of 1–50 µg/ml) and all were effective. Doses of 5 µg/ml killed all the worms when exposed for 24 h and over half of them when exposed for 2 h.

#### **7.4. Condition: acanthocephalan infections**

##### *7.4.1. Causative agents*

Polymorphid and neoechinorhynchid acanthocephalans.

Acanthocephalans can be readily distinguished from other helminths by their characteristically hooked, single proboscis. Like the nematodes, they have separate sexes and these can usually be determined at the cystacanth stage in the crayfish. Unlike the nematodes, acanthocephalans have no alimentary tract. Other than the single reported species of *Neoechinorhynchus* that has a couple of rows of a few hooks on the proboscis, the others are all polymorphids that have a swollen anterior trunk and a swelling in the proboscis. Some of these polymorphid juveniles in the crayfish have the appearance of a small pink grain of rice about 2 mm in diameter.

##### *7.4.2. Life cycle/life history*

Adult acanthocephalans typically parasitize the gut of vertebrate hosts and produce shelled larvae (acanthors) that are shed via the host's feces. Crayfish, small crustaceans or insects eat the larvae and become infected with a rather well-developed juvenile. Whether the crayfish can serve as an intermediate host or not has not been established for some species. Nevertheless, some species use the crayfish as a paratenic host, an ecologically but not biologically necessary host. Humans are more likely to become infected by eating poorly cooked polymorphids than neoechinorhynchids.

##### *7.4.3. Epizootiology*

Only a few acanthocephalans have been reported from crayfish in Australia (Johnston and Edmonds, 1948; O'Donoghue et al., 1990), Europe (von Siebold, 1835; Merritt and Pratt, 1964; Alderman and Polglase, 1988), and USA (Schmidt, 1973; Merritt and Pratt, 1964) (Table 3). O'Donoghue et al. (1990) reported *Polymorphus biziuræ* from *C. destructor* in South Australia, and Overstreet and Evans (unpublished data) have seen unidentified acanthocephalan infections in marron from Western Australia.

**Table 3.** Acanthocephalan parasites of freshwater crayfish

Species	Host	Geographic range	Other hosts	Prevalence in crayfish	Reference
<i>Polymorphus biziuræ</i>	<i>Cherax destructor</i>	South Australia	<i>Biziura lobate</i> (musk duck)	0.35% (n = 1948)	Johnston and Edmonds, 1948; O'Donoghue et al., 1990
<i>Polymorphus boschadæ</i>		Europe	Ducks and amphipods		von Siebold, 1835
<i>Southwellina dimorpha</i>	<i>Procarbarus clarkia</i>	Louisiana, USA	<i>Eudocimus albus</i> (white ibis)		Schmidt, 1973
<i>Neoechinorhynchus rutili</i>	<i>Pacifastacus trowbridgi</i>	Europe and USA	14 spp. of fish, ostracods	1.9% (n = 154)	Merritt and Pratt, 1964

The prevalence of infection is often low (e.g., 0.35% in 1948 specimens of *C. destructor* [O'Donoghue et al., 1990] and 1.9% of 154 *Pacifastacus trowbridgi* [Merritt and Pratt, 1964]). In some cases, the infections are paratenic, the crayfish acquiring the worm from feeding on an infected true intermediate host.

#### 7.4.4. Pathology

Acanthocephalans in crayfish often occur encapsulated, attached to the midgut, sticking into the haemocoel. They can also be in muscle, the haemolymph system, or elsewhere.

#### 7.4.5. Pathogen viability

Nothing is known about the viability of acanthocephalans within crayfish tissues.

### 7.5. Concluding remarks about metazoan parasites

Thorough parasitological surveys of crayfish for parasites are few (e.g., Soganders-Bernal, 1965; O'Donoghue et al., 1990), but there are numerous articles treating single or few metazoans. Most are digeneans. A high prevalence of infection of most of the parasites in crayfish is usually restricted to a specific geographic habitat, often highly restricted. Generally, information on viability of these parasites in crayfish tissues is not available. However, adequate freezing or heating kills parasites in seafood (Deardoff and Overstreet, 1991). Blast freezing of seafood to  $-40^{\circ}\text{C}$  for greater than 15 h or to  $-23^{\circ}\text{C}$  for at least 7 days is required by the US Food and Drug Administration (Deardoff and Overstreet, 1991).

## 8. Epibiont fouling organisms

### 8.1. Introduction

A range of ectocommensals or ectosymbionts from a number of different phyla infest the exoskeleton, including gills, of freshwater crayfish. Common groups include the peritrich ciliates, apostome ciliates, suctorian ciliates, temnocephalids, free-living nematodes, branchiobdellids, and ostracods. Algae, copepods, corixids, mites, rotifers, and polychaetes have

also been found in association with freshwater crayfish. Detailed information on these various organisms, their life histories, geographic location, and host species is provided in an excellent review by Alderman and Polglase (1988).

## 8.2. Condition: peritrich ciliate infestations

### 8.2.1. Causative agents

Common peritrich ciliates infesting freshwater crayfish include species in the genera *Epistylis*, *Cothurnia*, *Lagenophrys*, and *Zoothamnium*. Less well known are those species in the genera *Vaginicola*, *Pyxicola*, *Vorticella*, *Carchesium*, and *Sincothurnia*.

Peritrichs can be conveniently divided into those that possess a pseudochitinous lorica (e.g., *Cothurnia* and *Lagenophrys*) and those that do not (e.g., *Epistylis* and *Vorticella*). Complex staining and examination techniques are required to provide definitive identification of different genera and species (Corliss, 1979). However, common species groups such as those in *Cothurnia*, *Lagenophrys*, and *Epistylis* can be readily distinguished using light microscopical examination of unstained preparations.

All peritrich ciliates are characterized by the possession of a left-hand spiral of cilia leading to the mouth (or cytosome). Loricate ciliates possess shell-like structures called loricas that house the individual zooids. Aloricate species have zooids mounted on long stalks. Several publications on crayfish ectocommensals provide photographs, diagrams, and descriptions that can be used for identification purposes (e.g., Matthes and Guhl, 1973; Johnson, 1977; O'Donoghue et al., 1990; Evans et al., 1992). Reference to original descriptions in the published literature or to authoritative texts (e.g., Corliss, 1979) are required for definitive identification.

### 8.2.2. Life cycle/life history

When the host crayfish molts, new infestation occurs on the newly exposed epicuticle. The organisms attached to the discarded shell develop into telotroch stages then detach and actively seek a new attachment location. Transmission thus occurs through the aquatic environment. Confirmation of this mechanism of transmission is provided by Brown et al. (1993) who demonstrated transmission of an *Epistylis* sp. from *Orconetes rusticis* to *Orconetes virilis* during a severe outbreak of *Epistylis* infestation in a crayfish culture pond.

### 8.2.3. Epizootiology

Peritrich infestations of freshwater crayfish have been widely reported (Jensen, 1947; Nenninger, 1948; Hamilton, 1952; Krucinska and Simon, 1968; Sprague and Couch, 1971; Matthes and Guhl, 1973; Lahser, 1975; Johnson, 1977; Suter and Richardson, 1977; Mills, 1983, 1986; Kellicott, 1984; Scott and Thune, 1986; Herbert, 1987; Alderman and Polglase, 1988; Vogelbein and Thune, 1988; Owens and Evans, 1989; O'Donoghue et al., 1990; Evans et al., 1992; Thune, 1994; Boshko, 1995).

Freshwater crayfish species on which peritrichs have been described include *Astacoides granulimanus*, *Paranephrops zealandicus*, "Astacid crayfish," *Parastacoides tasmanicus* (Clamp, 1987, 1992, 1994), *C. destructor albidus*, *C. tenuimanus*, *C. quadricarinatus* (Kane, 1964, 1965; Mills, 1983, 1986; Villareal and Hutchings, 1986; Herbert, 1987; O'Donoghue et al., 1990;

Evans et al., 1992), *Engaeus fossor*, *E. cisternarius* (Suter and Richardson, 1977), *Procambarus clarkii* (Lahser, 1975; Scott and Thune, 1986; Vogelbein and Thune, 1988), *Procambarus zonangulus*, *Procambarus simulans simulans*, *Fallicambarus hedgpethi* (Lahser, 1975), *A. leptodactylus*, *A. fluviatilis*, *A. torrentium*, *Cambarus affinis* (Nenninger, 1948; Krucinska and Simon, 1968; Matthes and Guhl, 1973; Boshko, 1995), *C. immunis*, *C. immunis spinirostris*, *C. virilis*, *C. argillicola*, *C. bartoni*, and *C. bantoni robustus* (Hamilton, 1952), *Orconectes rusticus*, and *Orconectes virilis* (Brown et al., 1993).

Many peritrich ciliates exhibit a highly specific host-commensal relationship, suggesting that the term "symbiont" should be used to describe these organisms. An investigation of life stages of *Cothurnia variabilis* found in the gill chamber of *Pacifastacus gambeli* showed a synchrony between metamorphosis of the ectocommensal and the molt stage of the crayfish host (D'Eliscu, 1975). It is likely that similar synchrony occurs with other sessile peritrich ciliates and their respective hosts. The close interaction between the symbiont and its host may have implications for the likelihood of exotic peritrichs successfully colonizing related host organisms elsewhere. However, little research has been conducted in this area, most reports being restricted to documenting the occurrence of the organisms in a given host crayfish population rather than the experimental infestation of different crayfish species with a given symbiont.

#### 8.2.4. Pathology

Sessile peritrichs are found on the external surfaces, including the branchial chamber. Different species of peritrich ciliates show site specificity, some being found predominantly on the gills, some on the appendages and carapace, with others distributed widely over most of the body. The level of infestation by sessile peritrichs is dependent on the crayfish species, the aquatic environmental conditions and the stage of the molt cycle of the host. Matthes and Guhl (1973), Lahser (1975), O'Donoghue et al. (1990), and Evans et al. (1992) provided tabulated data on the prevalence of different peritrich ciliates on different crayfish hosts. These data provide evidence for variations in prevalence between different crayfish species as well as information on the range of species from different genera found in individual crayfish species.

Water quality has a significant effect on infestation levels, and turbidity is reported to be an excellent water quality indicator of potential peritrich infestation in commercial crayfish ponds (Scott and Thune, 1986). Infestation levels in farmed and wildstock crayfish of the same species have been shown to vary (O'Donoghue et al., 1990; Evans et al., 1992), probably as a result of variation in the aquatic environmental conditions.

Peritrichs are generally filter-feeding bacterivores (Corliss, 1979). Under eutrophic conditions, as sometimes occurring in aquaculture ponds, infestation levels increase (Scott and Thune, 1986). Some authors have suggested that if the peritrichs are localized in the gill cavity, dense populations may interfere with respiratory processes (Johnson, 1977; Villareal and Hutchings, 1986). Crayfish mortalities associated with heavy infestations of sessile peritrichs have been reported (Ninni, 1864; Kent, 1881–1882; Johnson, 1977; Villareal and Hutchings, 1986; Brown et al., 1993) and possible mechanisms of pathogenesis investigated (Vogelbein and Thune, 1988).

#### 8.2.5. *Pathogen viability*

External ciliates can be destroyed by treatment with toltrazuril (Baylox; 5–50 µg/ml for 24 h; Caughey, 1991). If treated for less time, a lower percentage of the individuals are killed, and species of different genera respond differently. For example, *Lagenophrys* sp. was not as affected as some of the stalked species. Weak formalin and acetic acid baths have also been used as control measures (O'Donoghue et al., 1990). High temperatures will destroy external ciliates but viability under low temperatures or in frozen product is uncertain.

### 8.3. *Condition: apostome ciliate infestations*

See Section 6.3.

### 8.4. *Condition: suctorian ciliate infestations*

#### 8.4.1. *Causative agents*

Suctorian ciliates from numerous genera, the most common being *Acineta* and less common genera including *Tokophrya*, *Podophora*, and *Opercularia*.

Suctorian ciliates are characterized by the possession of feeding (ingestatory) tentacles and a noncontractile stalk. Zooids of *Acineta* sp., observed by Vogelbein and Thune (1988), were 26–63 µm long with an apical width of 24–45 µm and a basal width of 16–32 µm. The stalk length ranged from 6 to 16 µm and the lobular macronucleus was in the apical one third of the zooid. *Acineta* spp. found in parastacid crayfish were larger, having an inverted conical-shaped body of 120–150 µm in length (O'Donoghue et al., 1990; Evans et al., 1992). Species in other genera have similar morphology but differ in the possession of a lorica and other diagnostic features (Corliss, 1979).

#### 8.4.2. *Life cycle/life history*

Motile larval stages ensure reinfestation of the crayfish host following shedding of the exoskeleton.

#### 8.4.3. *Epizootiology*

Suctorian ciliates have been described from numerous different crayfish species including *Procambarus clarkii* (Johnson, 1977; Scott and Thune, 1986; Vogelbein and Thune, 1988; Thune, 1994), *Procambarus simulans simulans* (Lahser, 1975), *A. leptodactylus*, *A. fluviatilis*, *A. torrentium*, and *Cambarus affinis* (Krucinska and Simon, 1968; Matthes and Guhl, 1973) and the *Cherax* species, *C. tenuimanus*, *C. destructor albidus*, and *C. quadricarinatus* (Kane, 1964, 1965; O'Donoghue et al., 1990; Evans et al., 1992). Transmission occurs through motile larval stages attaching to surfaces and developing into adults.

#### 8.4.4. *Pathology*

Suctorian ciliates are mainly found on external surfaces including the branchial chamber and feed primarily on free-swimming ciliate protistans (Hall, 1979; Sawyer et al., 1979). Suctorian ciliates are of little pathological significance to freshwater crayfish except when very high numbers are present in the gill chamber resulting in hypoxia.

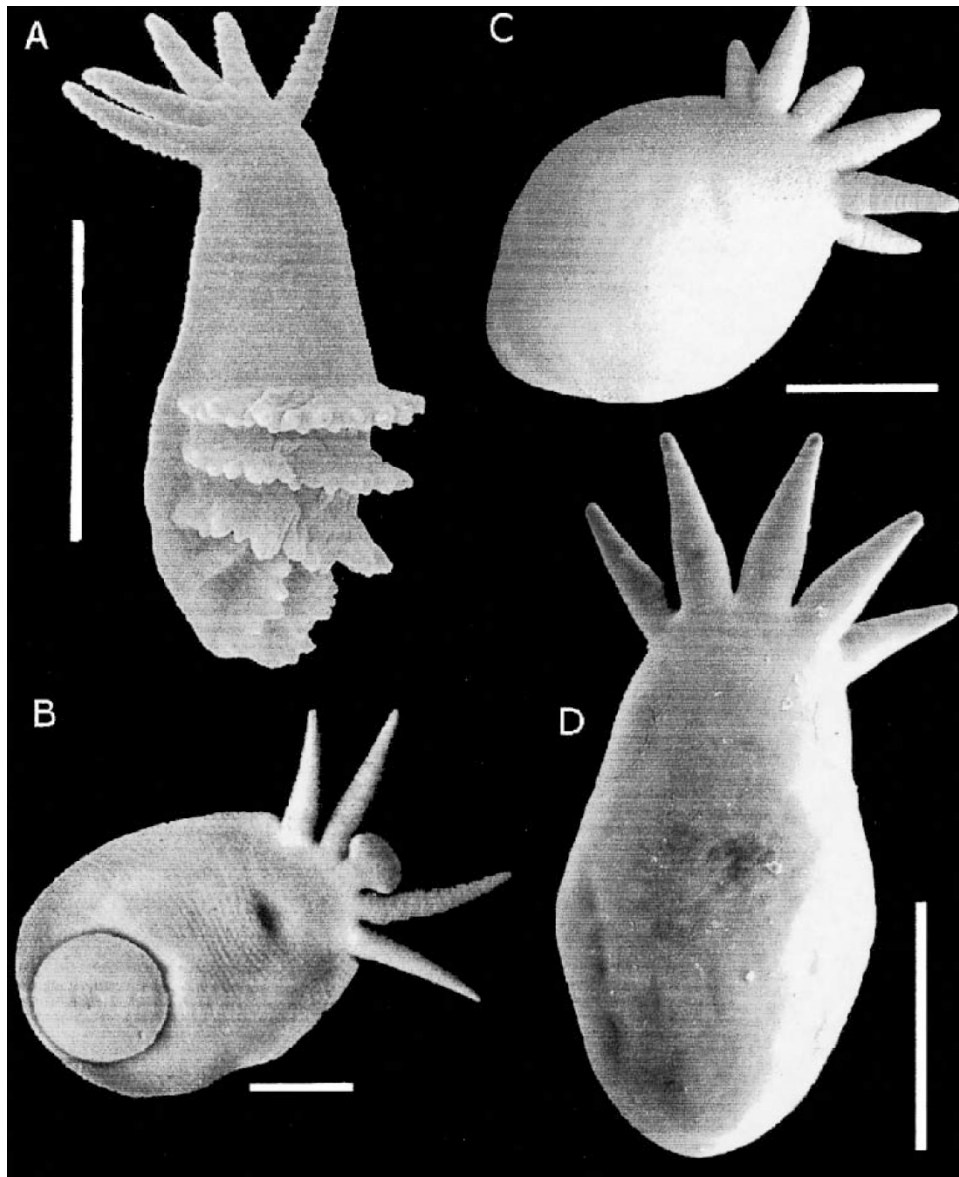
8.4.5. *Pathogen viability*

Information on viability under different processing conditions is lacking.

**8.5. Condition: temnocephalid infestations**

8.5.1. *Causative agents*

Temnocephalid ectosymbionts. Species include those in the common genera *Temnocephala*, *Diceratocephala*, and *Craspadella*. Other species include those in the genera *Notodactylus*, *Actinodactylella*, and *Decadidymus*. Temnocephalids are turbellarian flatworms. They are small organisms, generally oval or elliptical in shape (Fig. 14). Adult size varies, some species being small (e.g., *Craspadella yabba* 0.34–0.38 mm from posterior margin to tip of tentacles; Cannon and Sewell, 1995) and others being large (e.g., *Temnocephala minor*, 6–7 mm long; Cannon and Jennings, 1987). Adults possess anterior tentacles and a posterior ventral sucker. Most species have 5, 6, or 12 tentacles, although species belonging to the genera *Scutariella*, *Monodiscus*, and *Caridinicola* have only 2 tentacles.



**Figure 14.** Temnocephalans found in association with freshwater crayfish. (A) *Craspedella spenceri*; (B) *Temnomonticellia quadricornis*; (C) *Temnohaswellia* cf. *fasciata*; (D) *Temnocephala chilensis*. (Scale bar for A, B, and C = 500  $\mu\text{m}$ , for D = 200  $\mu\text{m}$ .) (Photograph supplied by Lester Cannon, Queensland Museum, Brisbane.)

#### 8.5.2. Life cycle/life history

Jones and Lester (1996) studied the transmission of the temnocephalid *Diceratocephala boschmai* following ecdysis in the redclaw, *C. quadricarinatus*, maintained in the laboratory. On average, 50% of the *D. boschmai* migrated successfully to the new exoskeleton of the host crayfish after ecdysis. Evidence has been presented to show that some species of



temnocephalids can survive and breed away from their hosts (Hickman, 1967; Jennings, 1971), and it has been suggested that a brief, free-living stage may compose part of the normal life cycle (Jennings, 1971).

#### 8.5.3. *Epizootiology*

Temnocephalids are mostly found in the tropics and in the southern hemisphere and have been reported from Australia, New Zealand, Central and South America, India, Sri Lanka, Madagascar, North America and the Balkans (Haswell, 1893; Jensen, 1947; Kane, 1964; Hickman, 1967; Jennings, 1971; Suter and Richardson, 1977; Williams, 1981; Gelder, 1983; Mills, 1983, 1986; Herbert, 1987; Cannon and Jennings, 1987; Cannon, 1991; O'Donoghue et al., 1990; Evans et al., 1992; Jones and Lester, 1992; Jennings et al., 1992; Cannon and Sewell, 1995). The geographic distribution of temnocephalids and specific associations with particular crayfish groups have been reviewed by Jensen (1947), Hickman (1967), and Jennings (1971).

Temnocephalids are described in a wide range of freshwater crayfish from Australia including the commercially important *Cherax* species, *C. tenuimanus*, *C. destructor albidus*, and *C. quadricarinatus*. There are reports of translocation of temnocephalids to other countries including Japan (Oki et al., 1995) and South Africa (Mitchell and Kock, 1988; Avenant-Oldewage, 1993), where they have been found infesting imported *C. tenuimanus* specimens. Of particular interest was the observation that an indigenous crab *Potamonautes warreni* in South Africa was also infested with the exotic ectosymbiont.

#### 8.5.4. *Pathology*

Temnocephalids are detected as ectocommensal organisms in the branchial chamber or on the body-proper of freshwater crayfish. Juveniles and adults browse over the host surface, feeding on annelids, small arthropods and cosymbiotic protists (Jennings, 1971; Cannon and Jennings, 1987). Temnocephalids have also been shown to consume the contents of crayfish eggs, although predation on intact crayfish eggs was rare (Jones and Lester, 1993). Oval- to round-shaped egg capsules are also often found adhering to the exoskeleton.

Temnocephalids possess anterior tentacles and a posterior ventral sucker, which is used for attachment. They occupy specific sites on the crayfish, some species being found predominantly in the gill chamber (e.g., *Craspadella spenceri*) and others on the carapace (e.g., *Temnocephala minor* and *D. boschmai*). Temnocephalid eggs are laid in thick capsules that are cemented onto the exoskeleton surface. Adults and eggs occupy the same anatomical site.

Aspects of the biology and pathogenicity of *D. boschmai*, an ectosymbiont of redclaw crayfish, *C. quadricarinatus*, have been studied by Jones and Lester (1993). Population densities on farmed crayfish varied seasonally, with relatively high numbers being observed in autumn and relatively low numbers in spring. A positive correlation between number of worms and host size was observed.

In the laboratory, *D. boschmai* exhibited low host specificity, with eggs being deposited on five different species of *Cherax* (*C. cuspidatus*, *C. depressus*, *C. destructor*, *C. tenuimanus*, and *C. quadricarinatus*). Worms were restricted to the carapace, none being found in the branchial chamber. Based on this observation and on the fact that the flatworms did not

attack healthy crayfish eggs, the authors concluded that the health of the crayfish was not significantly affected by infestations with *D. boschmai*. Jennings (1971) also concluded that temnocephalids were epicommentals with no evidence of parasitism, except for one European species of the family Scutariellidae, *Scutariella didactyla*, which apparently feeds on the host body fluids (Mrazeck, 1906).

Temnocephalid species that mainly inhabit the gill cavity and lay egg capsules on gill filaments (e.g., *Craspadella spenceri*) are likely to have a detrimental effect on the host. Detritus builds up around the egg capsules and provides a niche for bacteria and other epibionts (Jennings, 1971). This could affect respiration. However, despite reports of heavy infestations on farmed crayfish (Herbert, 1987), stock mortalities from temnocephalid infestation have not been reported. The major impact of these organisms is on the aesthetic quality of farmed crayfish. The presence of egg capsules on the external surface, usually on the underside of the tail, is unsightly and reduces the market value of the product. The egg capsules are difficult to remove, even with steaming or boiling.

#### 8.5.5. Pathogen viability

Adult temnocephalids can be removed from the crayfish host by bathing the animal in a rock salt bath (20–30 g/l) for approximately 10–15 min (Thorne, 1995). Aeration should be provided and the crayfish should be rinsed after the treatment. Formalin baths are also said to destroy temnocephalids (O'Donoghue et al., 1990). Neither of these treatments is effective against egg capsules. Egg capsules on the discarded exuvium were observed to remain viable and hatched up to 4 days after the exuvium was shed. There are little data available on the effect of freezing or cool storage temnocephalid survival.

#### 8.6. Condition: nematode infestations

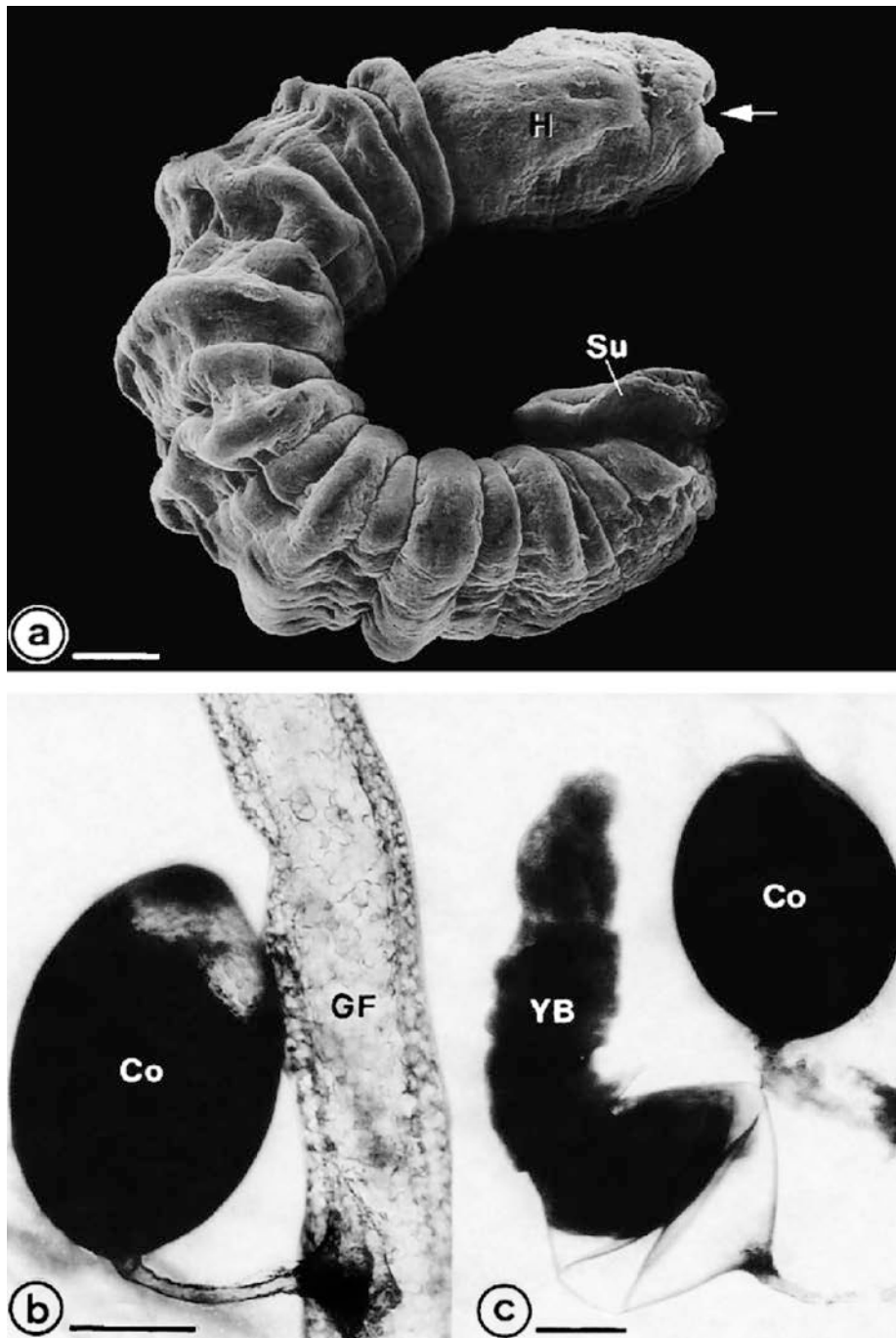
See Section 7.3.

#### 8.7. Condition: branchiobdellid infections and infestations

##### 8.7.1. Causative agents

Branchiobdellids belonging to numerous genera. The largest number of species of North American branchiobdellids belong to *Cambarinocola*. The only genus observed on European crayfish is *Branchiobdella* (Holt, 1965, 1968a).

Branchiobdellids are segmented worms, exhibiting a maximum of 14–15 segments, the first four of which are fused. They range in size from 1.0 to 7.0 mm as preserved specimens (Holt, 1975). A sucker with a circle of finger-like projections is present at the antenna end of the body and the posterior segments are modified to form another sucker (Fig. 15). In larger crayfish, eggs and young worms can be observed within cocoons that are attached to the exoskeleton, usually on the undersurface of the tail or on top of the rostrum (Johnson, 1977).



**Figure 15.** Branchiobdellids from *A. astacus*. (a) Scanning electron micrograph of adult branchiobdellid displaying head (H) with mouth (arrow), body segments, and posterior sucker (Su); (b) Cocoon (Co) attached to gill filament (GF); (c) Hatching young branchiobdellid (YB) from cocoon. (Scale bars = 100  $\mu\text{m}$ .) (From Vogt, 1999.)

### 8.7.2. *Life cycle/life history*

The life cycle of branchiobdellids is poorly understood. Eggs are laid in cocoons that are attached to the exoskeleton. Young worms commence development within the cocoons but relatively little is known of the developmental stages. Branchiobdellids have been observed away from the host (Young, 1966; Bishop, 1968; Holt, 1973b), but they are not thought to be capable of spending any appreciable length of time living independent of the host (Holt, 1975). Several authors have speculated that transmission occurs as a result of body contact between infested and noninfested crayfish (Holt, 1975; Thune, 1994).

### 8.7.3. *Epizootiology*

A wide range of crayfish species from North America, Europe, and East Asia are infested or infected by branchiobdellids (Goodnight, 1940; Hoffman, 1963; Pop, 1965; Holt, 1965, 1968a,b, 1973a,b, 1974, 1975; Bishop, 1968; Lahser, 1975; Simon, 1977; Johnson, 1977; Eng and Daniels, 1982; Vogt, 1999). No branchiobdellids occur in the southern hemisphere (Holt, 1975). Some branchiobdellids show low host specificity and are said to be able to exist on any crustacean of suitable size (Holt, 1975). A small number of species have been described from freshwater shrimp, crabs, and isopods, but their most common hosts are freshwater crayfish.

### 8.7.4. *Pathology*

Branchiobdellids are mostly found as epicommensals adhering to the external surfaces of crayfish. Some species are found on the gills (Vogt, 1999). Up to six different species have been observed on the body of a single host (Holt, 1975), and the specific anatomical location of a given species can vary seasonally and with the stage of development (Simon, 1977). Some species of branchiobdellids occupying the gill chamber have been described as ectoparasites and host defense responses comprising melanization of gill filaments at the point of attachment have been observed (Alderman and Polglase, 1988). Despite this evidence of a pathogenic effect on the crayfish host, there have been no confirmed reports of crayfish mortalities attributed directly to branchiobdellid infection or infestation. Furthermore, a recent laboratory study on the effect of the branchiobdellid *Cambarincola fallax* on growth and stamina of *Orconectes rusticus* failed to demonstrate any influence on growth rates or stamina of the host crayfish (Keller, 1992).

### 8.7.5. *Pathogen viability*

Branchiobdellids can be removed by boiling crayfish or by dipping crayfish in a salt solution (Johnson, 1977). Detailed studies of viability under conditions of reduced temperature or freezing have not been reported.

## 8.8. *Condition: ostracod infestations*

### 8.8.1. *Causative agents*

Ostracods of two subfamilies of the order Entocytheridae, the Entocytherinae from North and Central America, and the Notocytherinae from Australasia.

Ostracods are small crustacean epicommsals that inhabit the external surfaces and gills of freshwater crayfish. They exhibit a bivalved carapace that completely encloses the body. Antennae bearing long setae and other appendages normally protrude below the edge of the valves. Size ranges vary from approximately 1 to 3 mm.

#### 8.8.2. *Life cycle/life history*

The life cycle of ostracods is not fully understood. Eggs have been observed attached to setae of the gnathal appendages and other sites. Various instar stages are described (Hobbs, 1971; Walton and Hobbs, 1971). Experimental studies on ostracod reproduction showed that eggs are preferentially laid on slow molting, larger crayfish and that they can complete development on discarded exuvae.

#### 8.8.3. *Epizootiology*

Entocytherid ostracods have been described for a wide range of cambarid and parastacid crayfish from North and Central America and Australasia (Klie, 1931; Hoff, 1942; Hart and Hart, 1967, 1974; Hobbs et al., 1967; Hobbs and Hobbs, 1970; Hobbs, 1971; Lahser, 1975; Hobbs and Walton, 1976; Suter and Richardson, 1977; Eng and Daniels, 1982; Hobbs and Peters, 1989, 1993). They have been described from all genera of Australian crayfish (Hart and Hart, 1967) including wildstock and farmed *C. tenuimanus* (Evans et al., 1992). Two species have been reported on *C. destructor albidus*, *Notocyther syssitos*, and *N. mirrantia* (Mills, 1986). They have a low host specificity.

A few specimens of ostracod species infesting freshwater crayfish have been collected from the water of crayfish burrows (Klie, 1931; Hoff, 1942; Taliaferro and Salmons [pers. comm.] cited by Walton and Hobbs, 1971). These observations, supported by experimental studies (Young, 1971) suggest that ostracods are not obligate epicommsals and that they can survive away from the host. Transmission could therefore occur through the aquatic environment. Other modes of transmission including transmission during copulation of the hosts, or through direct host body contact, have been proposed (Walton and Hobbs, 1971).

#### 8.8.4. *Pathology*

Ostracods can be observed clinging to setae or in the crevices or grooves of the exoskeleton. Ostracods move freely around the setiferous areas, grazing on dietrital particles (Hobbs et al., 1967). Although regarded as having no direct adverse effects on the crayfish host, they can have an indirect adverse influence by harboring the cercarial stages of digenean trematodes that can infect the host (Alderman and Polglase, 1988).

Ostracod infestation levels vary with the stage of the molt cycle, the ostracod species, the crayfish species and the size of the host. Numbers are significantly reduced on animals that are heavily encrusted with foreign matter (Walton and Hobbs, 1971). Seasonal variations in population density have also been reported (Young, 1971). Evidence for a tendency for different species or life cycle stages being restricted to specific anatomical locations on the host has been presented (Walton and Hobbs, 1971).

#### 8.8.5. *Pathogen viability*

Information on viability under different environmental conditions or after disinfectant treatments is lacking but ostracods are unlikely to survive salt bath treatments and would be destroyed by adequate cooking regimes. Being epicommsals, they would also be effectively removed by processing procedures resulting in the removal of the exoskeleton.

### 8.9. *Condition: algal cuticular fouling*

#### 8.9.1. *Causative agents*

Cyanobacteria (blue-green algae) and Chlorophyta (green algae).

#### 8.9.2. *Epizootiology*

Exoskeleton fouling by species of cyanobacteria and green algae has been described in crayfish from North America (Lahser, 1975) and Australia (Evans et al., 1992). The North American study involved examination on 50 adult and juvenile crayfish of four species, *Procambarus simulans simulans*, *Procambarus clarkii*, *Procambarus zonangulus*, and *Fallicambarus hedgpethi*. Detailed identifications were performed and the presence of 4 species of cyanobacteria and 16 green algal species reported.

Unidentified filamentous algae have also been observed on the exoskeleton of *C. tenuimanus* during a health survey conducted on wild stock and cultured crayfish (Evans et al., 1992). The exoskeleton of 19% of animals examined was infested. Infestation levels were light and the organism was considered to be of no health significance.

#### 8.9.3. *Pathology*

Cyanobacteria and green algae are predominantly in the gill cavities of freshwater crayfish, although on occasions, specimens are observed on the carapace.

#### 8.9.4. *Pathogen viability*

No information is available on viability of these organisms.

### 8.10. *Condition: other epibiont infestations*

Species from seven other invertebrate groups are occasionally observed as symbionts or commensals of freshwater crayfish. These groups, along with representative reported species, are listed below.

#### 8.10.1. *Causative agents*

Corixids; eggs of *Ramphocorixa balanodis* (Abbott, 1912) and *Ramphocorixa acuminata* (Meyer, 1965).

Copepod infestation; *Nitocra divaricata* (Chappuis, 1923, 1926; Defaye, 1996).

Oligochaete infestation; *Hystricosoma chappusi* (Michaelsen, 1926; Moszynski, 1938) and *Phreodrilus* spp. (Goddard, 1909; Williams, 1980).

Polychaete infestation; *Stratiodrilus novaehollandiae*, *S. tasmanicus* (Haswell, 1913; Harrison, 1928; Cannon and Jennings, 1987).

Arachnid (mite) infestation; *Astacrotion molle* (Haswell, 1922; Viets, 1931a), *Astacopsiphagus parasiticus* (Viets, 1931b), *Lochmanella violacea*, *Parchalicharus alpinus*, *Limnohalacarus wackeri* (Viets, 1927), *Peza daps* (Harvey, 1990), and other (or possibly the same) unidentified species (Turner, 1926; Reck, 1942; Kane, 1964; Suter and Richardson, 1977).

Rhabdocoel flatworm infestation; *Didymorchis cherapsis* (Haswell, 1916; Williams, 1980; Cannon and Jennings, 1987); *Didymorchis* sp. (Evans et al., 1992).

Rotifer infestation; *Lepadella astacicola*, *L. branchiola*, *L. parasitica*, *L. borealis*, *L. lata*, *Cephalodella crassipes*, *Dicranophorus hauerianus* (Krucinska and Simon, 1968; May, 1989), *Mikrocodides* sp., *Monostyla* sp., and *Branchionius* sp. (Lahser, 1975).

Information on diagnostic features can be obtained from references at the end of this paper.

### 8.10.2. Epizootiology

**8.10.2.1. Corixids.** Corixids deposit eggs on the exoskeleton of freshwater crayfish (Overstreet, 1983). The numbers of eggs can become numerous if corixid populations are high and molting frequencies low (Thune, 1994). All reports of corixid associations with freshwater crayfish have been from the United States. Species of Corixidae are reported from Australia (Williams, 1980) but not in association with freshwater crayfish.

**8.10.2.2. Copepods.** Copepods are said to be well-known symbionts of freshwater crayfish (Alderman and Polglase, 1988). They are generally found in the gill chambers. Reports of copepod symbionts from freshwater crayfish have all been from European species of crayfish (Chappuis, 1923, 1926; DeFaye, 1996). There have been no reports of copepod infestations of Australian crayfish.

**8.10.2.3. Oligochaetes.** Oligochaetes are not commonly reported as symbionts or commensals of freshwater crayfish, having only been described on a few crayfish species from Europe (Michaelsen, 1926; Moszynski, 1938) and on *Euastacus* spp. (Goddard, 1909; Kane, 1964) and *C. destructor albidus* (Williams, 1980) from Australia. The polychaetes from European crayfish belong to the genus *Hystericosoma*, whereas those from Australian crayfish belong to the genus *Phreodrilus*. They are reported to occupy the external surfaces of the hosts (Alderman and Polglase, 1988).

**8.10.2.4. Polychaetes.** *Stratiodrillus* spp. are frequently observed in the gill chambers and on body surfaces of freshwater crayfish from Australia, Madagascar, and South America (Haswell, 1913; Harrison, 1928; Cannon and Jennings, 1987; Alderman and Polglase, 1988). Two species of the family Histriobdellidae occur in Australia, *S. novaehollandiae* and *S. tasmanicus* (Williams, 1980). Nutritional relationships of the polychaete *S. novaehollandiae* with three other ectosymbionts from two freshwater crayfish, *C. dispar* and *C. punctatus* in Queensland, Australia have been described (Cannon and Jennings, 1987) but few investigations appear to have been performed on life histories of polychaete ectosymbionts of freshwater crayfish.

**8.10.2.5. Mites.** A variety of mites are found in association with freshwater crayfish with reports from Australia (Haswell, 1922; Viets, 1931a,b; Reck, 1942; Kane, 1964; Suter and Richardson, 1977; Harvey, 1990), Germany (Viets, 1927), and Ohio (Turner, 1926). They are mostly found in the gill chamber although specimens have been observed on the carapace (Alderman and Polglase, 1988).

**8.10.2.6. Rhabdocoel flatworms.** There have been several reports of species of *Didymorchis* infesting crayfish of the genera *Cherax* from Eastern Australia and Queensland (Haswell, 1916; Williams, 1980; Mills, 1983, 1986; Cannon and Jennings, 1987) and Western Australia (Evans et al., 1992). No reports were found of dalyellioid rhabdocoels associated with crayfish from countries other than Australia. The flatworms are found predominantly on the gills and, rarely, on other external surfaces (Cannon and Jennings, 1987; Evans et al., 1992).

**8.10.2.7. Rotifers.** Rotifers have been observed on the external surfaces including the branchial cavity of crayfish from Eastern Europe and USA (Krucinska and Simon, 1968; Lahser, 1975; May, 1989) and Australia (Suter and Richardson, 1977; Evans et al., 1992). In her review of epizoic and parasitic rotifers, May (1989) stated that rotifers are commonly found in the branchial chambers of freshwater crayfish but that little is known about the nature of the relationship between crayfish and rotifers.

#### *8.10.3. Pathology*

The organisms listed above can be observed in wet mount microscopy of exoskeletal preparations including preparations of gills.

#### *8.10.4. Pathogen viability*

Remarks given in other sections on epibiont fouling organisms generally apply to the organisms listed above. Laboratory-based studies on most of these organisms are few and detailed information on viability is unlikely to be available in the published literature.

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