

## A Review of the Diseases of Cultured Penaeid Shrimps and Prawns with Emphasis on Recent Discoveries and Developments

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**Abstract** The development of the commercial culture of penaeid shrimps and prawns has been accompanied by the occurrence of diseases of infectious and noninfectious etiologies. Many of the important penaeid diseases are caused by organisms that are part of the normal microflora and fauna of penaeids. These organisms are opportunistic pathogens that cause disease only under conditions that favor them over the host. Many organisms in this category are ubiquitous, and most have been recognized and/or reported from each of the major penaeid culture areas of the world. Included among this category of pathogens are the filamentous bacteria *Leucothrix mucor*, *Flexibacter* sp. and *Cytophaga* sp. (agents of filamentous gill and surface fouling diseases); the peritrich protozoans *Zoothamnium* sp., *Epistylis* sp., and *Vorticella* sp. (surface epibionts that cause protozoan gill disease and surface fouling diseases), the invasive bacteria *Vibrio alginolyticus* and *V. parahaemolyticus* (agents of various bacterial disease syndromes); and the fungi *Lagenidium callinectes*, *Sirolopidium* sp., and *Fusarium solani* (agents of the most common fungus diseases of penaeids).

Among the most important disease-causing agents are the penaeid viruses. These penaeid viruses may once have been limited in their geographic distribution in wild stocks, but they have become widespread in penaeid culture facilities. With the advent of commercial penaeid hatcheries, the shipment of broodstock and postlarvae from these culture facilities to others in different geographic regions has often resulted in the spread of these agents outside their normal range in wild populations. Included in this category of the penaeid viruses are the baculoviruses: *Baculovirus penaei* (BP), *P. monodon* baculovirus (MBV), baculoviral midgut gland necrosis virus (BMN); the hepatopancreatic parvo-like virus (HPV); the probable picornavirus infectious hypodermal and hematopoietic necrosis virus (IHHNV), and a reo-like virus in *P. japonicus*.

The final group of important diseases of cultured penaeids are the nutritional, physical, and toxic disease syndromes. The ascorbic acid deficiency syndrome called "black death" is the best understood nutritional disease of penaeids. Among the physical diseases occurring in penaeid culture, gas bubble disease and tail cramp are probably the most common. Important toxic disease syndromes include aflatoxicosis and red disease (which may be due to mycotoxins); hemocytic enteritis (due to certain species of filamentous blue-green algae, especially *Schizothrix calcicola*) and toxic syndromes due to toxic algal blooms.

There are five areas of research that should receive emphasis in the next several years in penaeid disease research: 1) Appropriately equipped laboratories in each of the major penaeid culture areas should identify and catalog those diseases occurring in culture facilities in their region; 2) Penaeid diagnostic laboratories should use, or strive to develop for general use, "standardized" diagnostic procedures whenever possible, especially for highly infectious agents such as the penaeid viruses; 3) Penaeid cell culture methods for primary cultures or cell lines must be developed to aid in the development of much needed rapid, sensitive diagnostic tests for the penaeid viruses; 4) Improved methods of disease prevention, control, or chemotherapy are needed for many of the penaeid diseases now adversely affecting the penaeid culture industry; and 5) Approval is needed from those government agencies (such as the U.S. Food and Drug Administration and the Environmental Protection Agency) for the drugs and chemicals used as chemotherapeutics in penaeid culture that may pose a health risk to humans.

### Introduction

The rapid growth of the penaeid shrimp culture industry has been accompanied by an increased awareness of the negative impact of disease on the industry. The development of the industry has been accompanied by the occurrence of diseases of infectious and noninfectious etiologies. The relative importance of disease is somewhat dependent on the type of culture system employed. Neal (1973) defined the two general methods of shrimp culture that were practiced a decade ago as intensive and extensive. Today these two types of culture systems may be subdivided into three general types of culture systems: Systems that raise shrimp in high-density, intensively managed tanks and raceways are defined as intensive culture systems. Systems producing moderate densities of shrimp in cages, ponds or tanks are

considered to be semi-intensive, while extensive culture is the culture of shrimp in low-density ponds or pens in which little or no management is exercised or possible. Generally, culture systems that include a hatchery for "seed" (post-larvae) production are semi-intensive or intensive, whereas those relying on "wild seed" typically fall into the extensive or semi-intensive class. It is in most semi-intensive and intensive culture systems that the recognition, prevention, and treatment of disease is possible whereas in extensive and many semi-intensive systems, treatment of diseases is impractical even if they are diagnosed. Furthermore, except for certain types of parasitic diseases, it is the very nature of intensive and semi-intensive culture systems (i.e., high shrimp density per unit volume of water used) that encourages the development and transmission of many shrimp diseases. The

same economic incentives for using semi-intensive and intensive culture systems dictate that disease be understood and controlled.

Knowledge of the diseases of penaeid shrimps and prawns has been reviewed a number of times within the past 12 years (Overstreet, 1973, 1982; Sindermann, 1974; Johnson, 1975a, 1978; Lightner, 1977; Couch, 1978, 1983). This review emphasizes recent developments and recent discoveries in shrimp pathology made since the most recent review.

## Infectious diseases

### Virus diseases

Six virus-caused diseases of cultured penaeids have been reported (Table 1) and several additional diseases have been noted to have associated with them virus-like or chlamydia-

**Table 1.** Penaeid viruses and their known natural and experimentally infected hosts<sup>a</sup>.

Subgenus	BP	MBV	BMN	IHHNV	HPVREO
<i>Litopenaeus:</i>					
<i>Penaeus vannamei</i>	++			+	
<i>P. stylirostris</i>	+			++	
<i>P. setiferus</i>	+			+(e)	
<i>Penaeus:</i>					
<i>P. monodon</i>		++		++	+
<i>P. esculentus</i>					++
<i>P. semisulcatus</i>		+		+	+++
<i>Fenneropenaeus:</i>					
<i>P. merguensis</i>		++			+++
<i>P. orientalis</i>					++
<i>Farfantepenaeus:</i>					
<i>P. japonicus</i>			+++	+(e)	++
<i>P. aztecus</i>	+++			+(e)	
<i>P. duorarum</i>	+++			+(e)	
<i>P. kerathurus</i>		+(?)			
<i>P. marginatus</i>	+++				

<sup>a</sup>Abbreviations:

BP = *Baculovirus penaei*

MBV = *P. monodon* baculovirus

BMN = Baculoviral midgut gland necrosis

IHHN = Infectious hypodermal and hematopoietic necrosis

HPV = Enteric parvo-like virus

REO = Reo-like virus

+ = Infection observed in species, but no signs of disease.

++ = Infection may result in moderate disease, mortalities.

+++ = Infection usually results in serious epizootic with high mortality rate.

e = Experimentally infected; natural infections not yet observed.

like structures (Table 2). Included among the penaeid viruses causing disease in penaeids and documented in the literature are: the three baculoviruses *Baculovirus penaei* or BP (Couch, 1974), baculoviral midgut gland necrosis or BMN (Sano et al., 1981), and *Penaeus monodon* baculovirus or MBV (Lightner and Redman, 1981); the probable picornavirus infectious hypodermal and hematopoietic necrosis virus or IHHNV (Lightner et al., 1983a); the small DNA-containing virus named hepatopancreatic parvo-like virus or HPV (Lightner and Redman, in press a); and a reo-like virus in the hepatopancreas of *P. japonicus* (Tsing and Bonami, 1984).

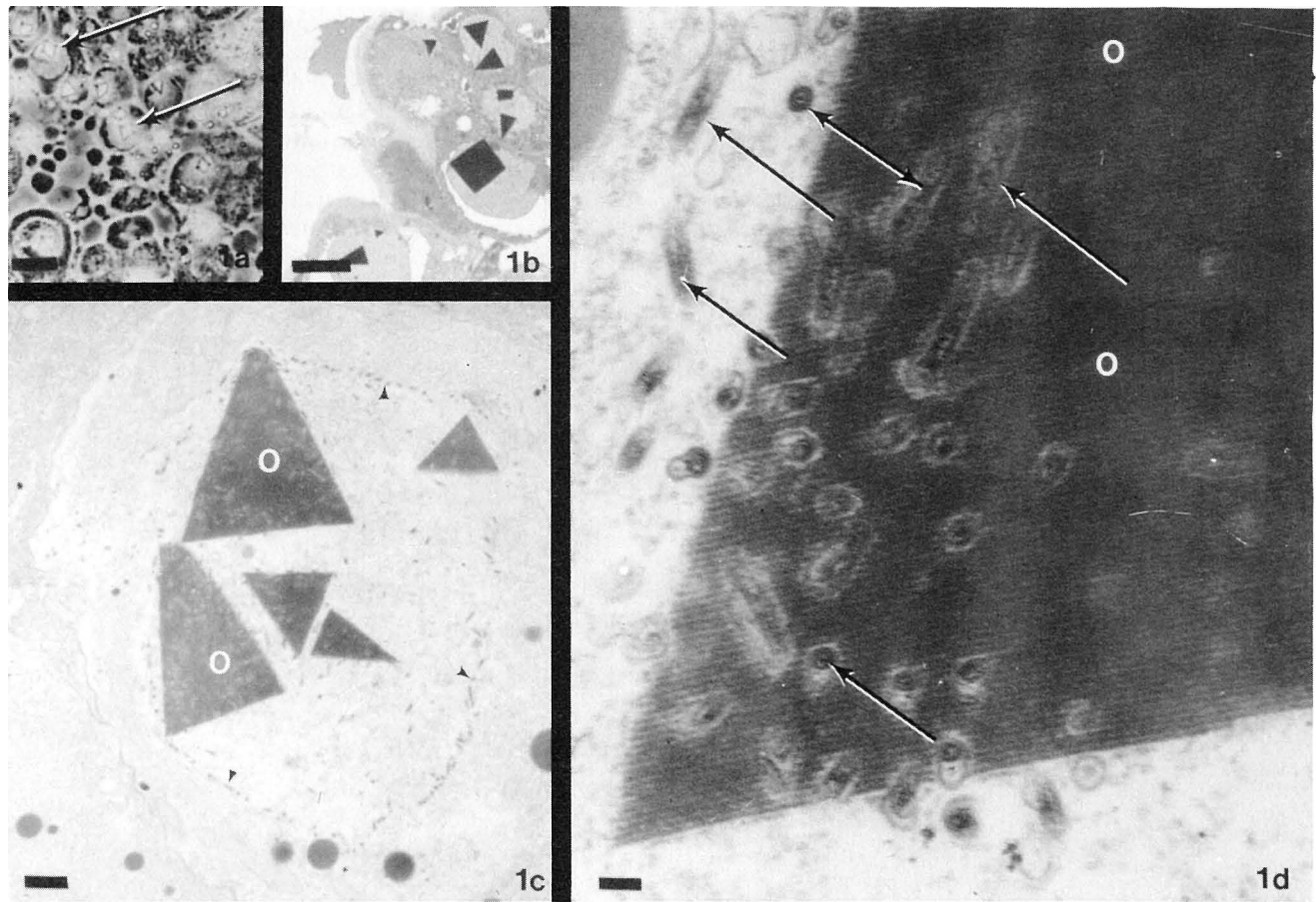
**Baculoviruses.** The three known penaeid baculoviruses infect the epithelial cells of the hepatopancreas of protozoal through adult life stages and the midgut epithelium of larvae and postlarvae. Baculovirus infections may result in disease in cultured penaeids that is accompanied by high mortality rates. In hatcheries, BP and BMN often cause serious epizootics in the larval and early postlarval stages of their principal host species (Tables 1 and 3) (Couch, 1981; Sano et al., 1981), and BP may cause disease and mortalities in juvenile and Subadult animals (Couch 1981). Disease epizootics due to MBV in hatchery-reared *P. monodon* are known to occur from late postlarval (PL<sub>25</sub> to PL<sub>50</sub>) through the juvenile and adult life stages, although the most serious losses have been observed in the late postlarval stages (Lightner et al., 1983c).

The geographic distribution of these baculoviruses in cultured penaeid shrimp suggests that some are problems to shrimp culturists only in those areas where the virus is apparently enzootic in local wild populations. This appears to be the case for BMN which has only been observed in *P. japonicus* in hatcheries in Japan (Table 3). MBV and BP, however, have been documented to have been introduced into new geographic regions by the transfer of infected postlarvae or broodstock to areas outside the normal range of the host species (Table 3).

Patent acute BP and MBV infections may be readily diagnosed by demonstration of their characteristic occlusion bodies (specialized inclusion bodies of type A baculoviruses) in either wet mounts or histological preparations of the hepatopancreas and midgut (Lightner et al., in press a,b). BP occlusions are distinctive tetrahedral bodies easily detected by bright field or phase microscopy in unstained wet mounts of tissue squashes (Fig. 1), while MBV occlusions are spherical and therefore difficult to distinguish from lipid droplets, secretory granules, etc. The use of a stain like 0.1% aqueous malachite green in preparing wet mounts for MBV diagnosis aids in demonstration of the occlusions. Presumably, the pro-

**Table 2.** Additional penaeid diseases of possible viral or chlamydial etiology, and possible orphan viruses observed in penaeids.

Agent, condition or disease	Host species	Organ	Associated with disease	Reference
IHHN-like inclusions	<i>Penaeus japonicus</i>	HCO & foregut	No	Brock (unpub.)
Picorna or Parvovirus	<i>P. aztecus</i>	Heart	No	Foster et al., 1981
Togavirus	<i>P. duorarum</i>	HP	No	Lightner (unpub.)
Picorna or Parvovirus	<i>P. japonicus</i>	Whole body	Yes	Bonami (pers. comm., Sept. 1984)
Chlamydia-like agent	<i>P. japonicus</i>	HP	Yes, with BMN	Lightner (unpub.)



**Fig. 1.** *Baculovirus penaei* Couch (BP): 1a) Intranuclear polyhedral occlusion bodies (arrows) in a wet mount of the hepatopancreas of a pink shrimp *Penaeus duorarum* (photo courtesy of J. A. Couch, U.S.E.P.A., Gulf Breeze, FL),  $\times 525$ . Bar is  $10\mu\text{m}$ ; 1b) BP occlusion bodies in a plastic histological section of the hepatopancreas of a postlarval brown shrimp *P. aztecus* (toluidine blue),  $\times 800$ . Bar is  $10\mu\text{m}$ ; 1c) TEM of a BP-infected *P. vannamei* hepatopancreatocyte, showing occlusion bodies (O) and virions (arrowheads),  $\times 5,400$ . Bar is  $10\mu\text{m}$ ; 1d) Higher magnification TEM of a BP occlusion body (O) and virions in *P. vannamei*. Rod-shaped BP virions (arrows) are present free in the karyoplasm and occluded within the tetrahedral occlusion body,  $\times 54,000$ . Bar is  $100\text{nm}$ .\*

tein making up the occlusion absorbs the stain more rapidly than do most of the host tissue components, making the occlusions distinct within a few minutes (Fig. 2). BP and MBV occlusions distinct within a few minutes (Fig. 2). BP and MBV occlusion bodies in histological preparations appear as prominent eosinophilic (with H&E), usually multiple occlusion bodies within the hypertrophied nuclei of hepatopancreatic tubules or midgut epithelial cells.

Unlike BP and MBV, which are Type A baculoviruses because they produce occlusion bodies, BMN is a Type C baculovirus that does not produce an occlusion body (Fig. 3). Hence, diagnosis of BMN infections is dependent upon the clinical signs of the disease, histopathology and transmission electron microscopic (TEM) demonstration of the baculovirus in affected hepatopancreatocytes (Lightner et al., in press b). Sano et al. (1983) have developed a rapid fluorescent antibody test for BMN that reportedly simplifies the diagnosis of BMN.

\*For all figures, unless otherwise noted, wet mounts are unstained; histological sections are stained with hematoxylin and eosin; TEM sections are stained with lead citrate and uranyl acetate, and SEM preparations are coated with gold.

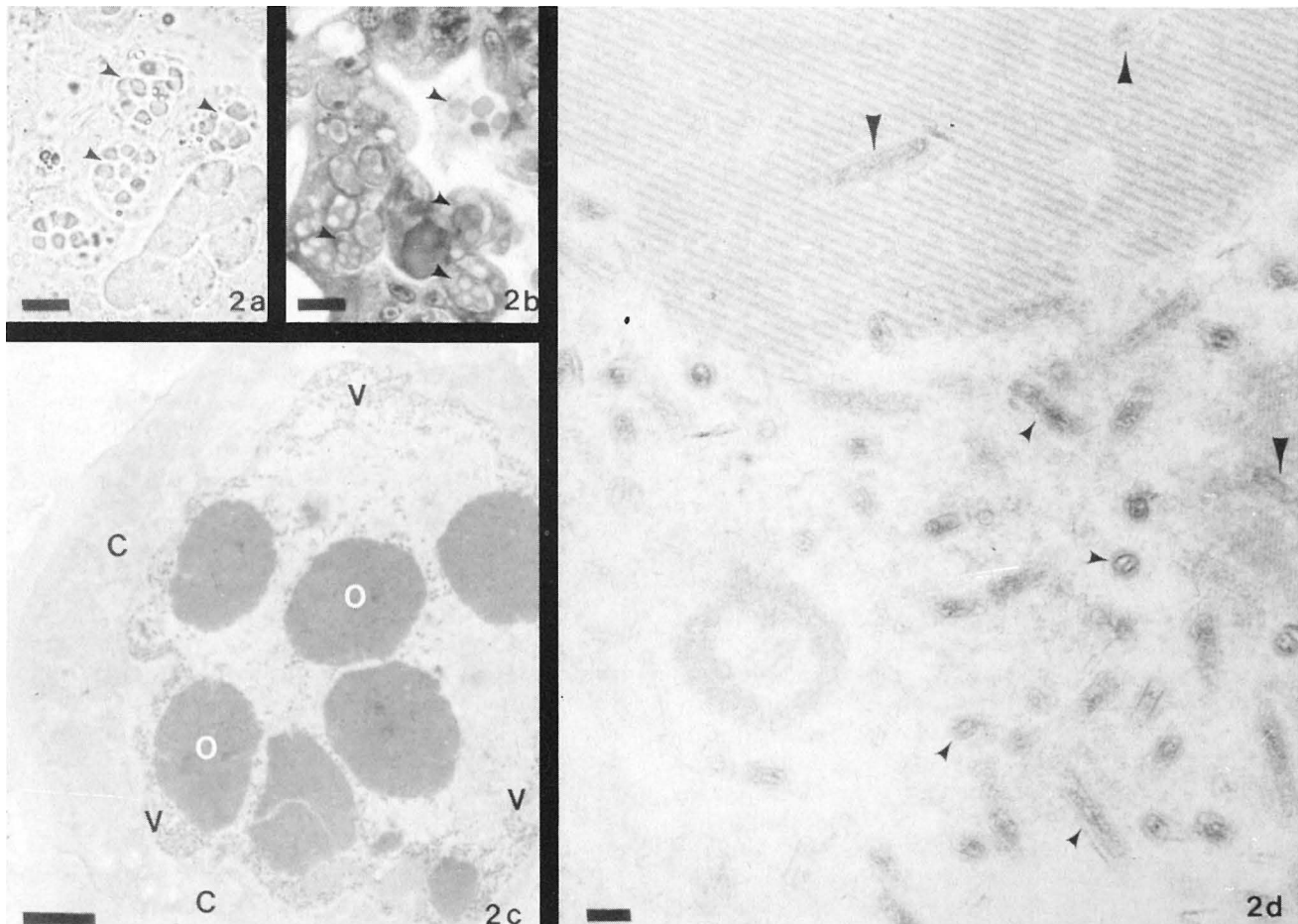
The cytopathology of BP, MBV, and BMN is generally similar when studied by light microscopy, differing principally by the lack of occlusion bodies in BMN. Often the affected hepatopancreatocyte nuclei have a peripherally displaced compressed nucleolus and marginated chromatin, giving affected nuclei a "signet ring" appearance (Figs. 1-3), even before occlusion bodies become well developed. Brown and Brenn histologic gram stain (Luna, 1968), although not specific for baculovirus occlusion bodies, tends to stain occlusions more intensely than the surrounding tissue, aiding in demonstrating their presence in low-grade infections.

TEM of BP and MBV-infected cells shows large numbers of rod-shaped baculovirus particles both free and occluded within the proteinaceous crystalline matrix of the occlusion body (Figs. 1 and 2), but only free virus in the nuclei of BMN-infected hepatopancreatocytes (Fig. 3).

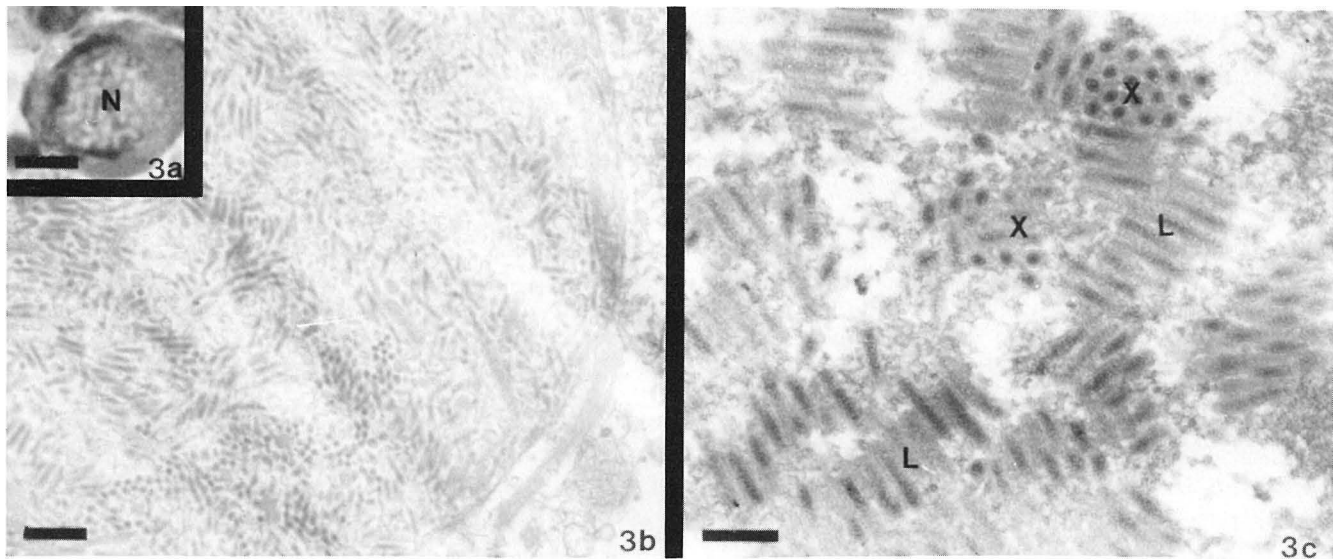
**IHHN virus.** This probable picornavirus (Fig. 4), named IHHNV for infectious hypodermal and hematopoietic necrosis virus, was first recognized in 1981 in Hawaii, in populations of cultured *P. stylirostris* that had been imported from a number of commercial penaeid hatcheries (Lightner et al., 1983a, 1983b). Since its discovery in *P. stylirostris*, IHHNV

has been found to infect a variety of other penaeid species either in natural infections or in experimentally-induced infections (Table 1). IHHN causes serious epizootics in intensively or semi-intensively reared *P. stylirostris*, with accumulative mortalities typically exceeding 90% of the affected populations within 14 to 21 days of onset in 0.05 to 2 g juveniles (Lightner et al., 1983a, 1983b). IHHN has also been documented to cause disease and serious epizootics in larger juvenile and adult *P. stylirostris* and in juvenile and adult *P. monodon* reared in intensive or semi-intensive culture systems (Brock et al., 1983). While IHNV has been shown to infect and to be carried asymptotically by *P. vannamei* (Lightner et al., 1983b; Bell and Lightner, 1984), significant mortalities due to IHNV infection in *P. vannamei* have not been documented. However, more study of IHNV disease in *P. vannamei* may indeed show that under stressful culture conditions, some mortality losses and/or reduced growth rates may occur (Lightner, unpub.).

IHNV has been detected in penaeid shrimp sampled from a number of shrimp culture facilities located in widely separated geographic locations (Table 4; Bell and Lightner, in press). These observations suggest that IHNV has become widely distributed in penaeid culture facilities (Bell and Lightner, in press; Table 4) probably as a result of the difficulty of detecting infection by the virus in asymptomatic carrier hosts such as *P. vannamei* or because losses due to the virus in pond-reared stocks are difficult to detect. IHNV is a disease of juvenile or older shrimp; apparently it does not adversely affect the larval or postlarval stages and, hence, its effect does not occur in hatcheries where it would be readily detected. Instead, IHNV produces its most serious epizootics in (*P. stylirostris* and probably *P. monodon*) shrimp of 0.05 to 2 g, the size by which shrimp have typically been moved to nursery or grow-out ponds. Water turbidity and the small shrimp size at this time in the life cycle makes detection of the disease in extensive or semi-intensive cul-



**Fig. 2.** *P. monodon*-type baculovirus (MBV): 2a) Intranuclear occlusion bodies in a wet mount of an MBV-infected hepatopancreas of a postlarval *P. monodon*. Multiple spherical occlusion bodies (arrowheads) are present in the hypertrophied nuclei of five of the cells shown (malachite green stain),  $\times 5,800$ . Bar is  $1 \mu\text{m}$ ; 2b) Histological section of a *P. monodon* hepatopancreas that is heavily infected by MBV. Multiple occlusion bodies (arrowheads) are present within hypertrophied nuclei,  $\times 600$ . Bar is  $10 \mu\text{m}$ ; 2c) TEM of an MBV-infected hepatopancreatocyte. Conspicuous occlusion bodies (O) and masses of free virions (V) are present in the hypertrophied nucleus, that is surrounded by a thin ring of cytoplasm (C) that is made dense by numerous free ribosomes,  $\times 4,680$ . Bar is  $2 \mu\text{m}$ ; 2d) High magnification TEM of MBV virions that are free in the karyoplasm (small arrowheads) or occluded (big arrowheads) within the protein matrix of occlusion bodies (O),  $\times 59,800$ . Bar is  $100 \text{ nm}$ .



**Fig. 3.** Baculoviral midgut gland necrosis (BMN): 3a) Histological section of the hepatopancreas of a postlarval *P. japonicus* with BMN, showing a hypertrophied hepatopancreatocyte nucleus (N). Occlusion bodies are not present in BMN-infected cells,  $\times 1,520$ . Bar is  $5\mu\text{m}$ ; 3b) TEM of a BMN-infected hypertrophied nucleus. Virions nearly fill the nucleus,  $\times 16,800$ . Bar is  $0.5\mu\text{m}$ ; 3c) Higher magnification of packets of rod-shaped BMN virions in cross-section (X) and longitudinal section (L),  $\times 39,600$ . Bar is  $250\text{nm}$ .

**Table 3.** Range of the penaeid baculoviruses in captive and cultured host species.

Virus	Host species	Geographic location	Reference
BMN	<i>Penaeus japonicus</i>	Japan	Sano et al., 1981
BP	<i>P. aztecus</i> , <i>P. duorarum</i> , <i>P. setiferus</i>	Florida, Mississippi	Couch, 1981; Overstreet, 1978
	<i>P. vannamei</i> , <i>P. stylirostris</i>	Panama, Costa Rica, Ecuador, Texas*	Lightner 1983; Lightner (unpub.)
	<i>P. marginatus</i>	Hawaii	Brock (in press)
MBV	<i>P. monodon</i>	Philippines, Taiwan, Malaysia, Singapore, Mexico*, Hawaii*, Tahiti*	Lightner et al., 1983c; Lightner (unpub.)
	<i>P. merguensis</i>	Singapore, Malaysia	Brock et al., 1983; Lightner (unpub.)
	<i>P. kerathurus</i>	Italy	G. Bovo (pers. comm., 1984)
	<i>P. semisulcatus</i>	Persian Gulf	Lightner (unpub.)

\*Denotes known example of the introduction of virus to a new geographic region with transfer of infected host species.

ture systems difficult. Complicating the disease further was the discovery that penaeid shrimp surviving IHHNV infections become carriers of the virus for life and pass the virus on to their offspring (Lightner et al., 1983b).

Diagnosis of infection by IHHNV is dependent upon histological demonstration of prominent eosinophilic (with H&E), Feulgen-negative intranuclear inclusion bodies (Fig. 4a) within chromatin-marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of foregut and hindgut, nerve cord, and nerve ganglia) and mesodermal origin (hematopoietic organs, antennal gland tubule epithelium, mandibular organ, connective tissue, and striated muscle). Usually the midgut, midgut caeca and the hepatopancreas (endoderm-derived tissues) are

**Table 4.** Known geographic distribution of IHHNV in cultured penaeid shrimp.

Host species	Culture locations positive for IHHNV
<i>Penaeus stylirostris</i>	Hawaii, Tahiti, Florida,
<i>P. vannamei</i>	Texas, Cayman Islands,
<i>P. monodon</i>	Israel, Panama, Costa Rica,
<i>P. semisulcatus</i>	Belize, Ecuador, Philippines,
<i>P. japonicus</i> (exp.)*	Singapore, and Guam.
<i>P. aztecus</i> (exp.)	Probable: Taiwan, Brazil, France,
<i>P. duorarum</i> (exp.)	Jamaica, and Honduras.
<i>P. setiferus</i> (exp.)	

\*"exp." denotes experimentally induced laboratory infection by the virus. Natural infections are not known to occur in species.

unaffected, except in severe cases where hepatopancreatic involvement has been observed. These inclusions match closely the characteristics of the Type A intranuclear inclusion body class described by Cowdry (1934). Basophilic chromatin strands are occasionally visible by light microscopy within IHHN intranuclear inclusion bodies. These chromatin strands are a prominent feature of IHHN intranuclear inclusion bodies by TEM (Fig. 4b). IHHN intranuclear inclusion bodies are common early in acute infections, later decreasing in number, and are followed by necrosis and inflammation of target tissues. Affected cells may also have highly vacuolate cytoplasm and small cytoplasmic basophilic inclusions (Fig. 4c). Although the prominent intranuclear inclusions present in shrimp infected with IHHNV are evidence of nuclear involvement, assembly of the virus occurs in the cytoplasm of affected cells (Fig. 4d). The size of the virus (17 to 26 nm in tissue sections and 20 to 22 nm in purified preparations), its morphology, and its replication within the cytoplasm support the tentative classification of IHHNV with the picornaviruses.

**HPV.** This probable parvovirus named HPV, or hepatopancreatic parvo-like virus (Fig. 5), was first recognized in cultured *P. merguensis* in Singapore and Malaysia in 1983 (Lightner and Redman, in press a). HPV (or a very similar agent) was subsequently recognized in four additional penaeid species (*P. orientalis*, *P. semisulcatus*, *P. esculentus*, and presumed *P. monodon*) in either captive wild populations or in cultured populations (Table 5). Individual shrimp with HPV displayed nonspecific signs including poor growth rate, anorexia, reduced preening activity, increased surface fouling, and occasional opacity of tail musculature. Mortalities accompanied by these signs occurred during the juvenile stages, after apparently normal development through the larval and postlarval stages. Accumulative mortality rates in HPV epizootics in *P. merguensis* and *P. semisulcatus* reached as high as 50% to 100%, respectively, of the affected populations within four to eight weeks of disease onset.

**Table 5.** Known geographic distribution of HPV in captive and cultured penaeid shrimp.

Host species	Geographic location	Reference
<i>Penaeus merguensis</i>	Singapore	
<i>P. orientalis</i>	Qingdao (Yellow Sea region), China	Lightner and Redman (in press)
<i>P. semisulcatus</i>	Persian Gulf	
<i>P. monodon</i>	Philippines	
<i>P. esculentus</i>	Queensland, Australia	Paynter et al. (in press)

The principal lesion in HPV disease, common to all affected species, is a necrosis and atrophy of the hepatopancreas, accompanied by the presence of large prominent basophilic, PAS-negative, Feulgen-positive intranuclear inclusion bodies in affected hepatopancreatocytes (Fig. 5a). These inclusion bodies are diagnostic for HPV, and presumably developed from small eosinophilic intranuclear bodies that were also present in the affected tissues. Electron microscopy of

affected hepatopancreatocytes revealed aggregations of 22 to 24 nm diameter particles within the electron-dense granular inclusion body ground substance (Fig. 5b). The virus-like particle size and morphology, the close association of the nucleolus with the developing inclusion body, and the presence of intranuclear bodies within developing inclusion bodies are similar to cytopathological features reported for parvovirus infections in insects and vertebrates.

**Reo-like virus.** A reo-like virus was present in large viral areas in the cytoplasm of hepatopancreatic R-cells of diseased laboratory-reared *P. japonicus* from the Mediterranean city of Palavas in France (Tsing and Bonami, 1984). Purified virions were non-enveloped, icosahedral particles of about 60 nm in diameter. The disease was reproduced in healthy *P. japonicus* by inoculation with purified virus or by feeding animals pieces of hepatopancreas from infected shrimp. Disease developed slowly in reo-like virus-exposed animals, requiring about 45 days to develop. Secondary infections by agents such as *F. solani* were common in reo-like virus-infected *P. japonicus* (Tsing and Bonami, 1984).

**General procedures for virus screening.** Three basic diagnostic procedures have been developed for screening penaeid shrimp for virus infections: 1) direct samplings for microscopic (wet mount) examination and/or histopathology; 2) enhancement of infection followed by microscopic examination and/or histopathology; and 3) bioassay of a suspect shrimp population with a sensitive indicator species followed by sampling and histopathology (Lightner et al., in press b).

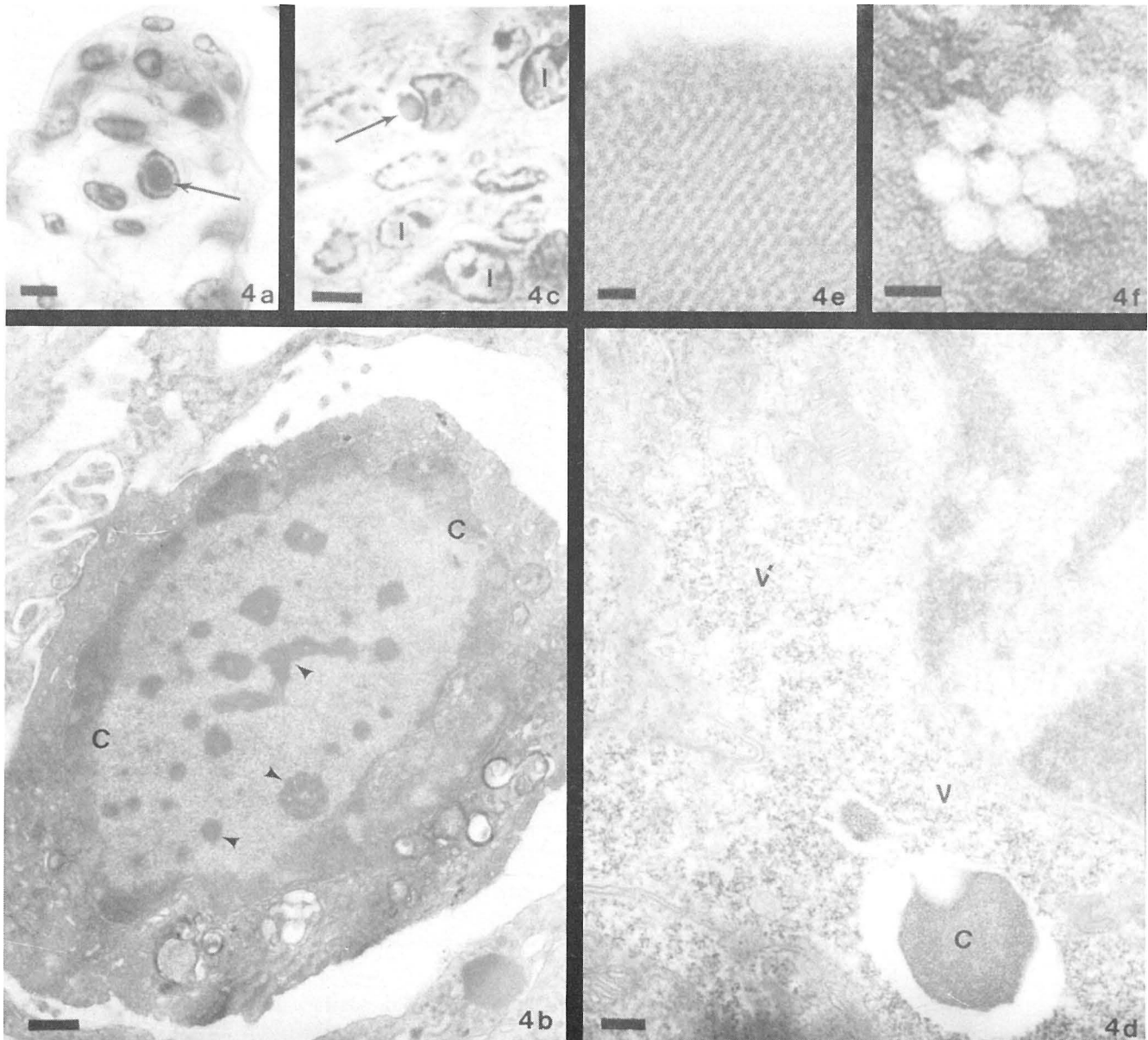
Nonrandom samples of shrimp are selected in direct sampling procedure from culture tanks, ponds, or cages and examined directly for signs of BP or MBV in wet mounts, or they may be preserved in Davidson's AFA or 10% buffered formalin (Humason, 1967) for histological evaluation. The sensitivity of this procedure is limited, and it will only demonstrate shrimp with viral infections that are acute or subacute in a population with a high incidence rate. We have been able to diagnose IHHN, BP, MBV, and HPV with direct samples, but such samples have also produced false negative diagnoses on populations later shown by enhancement or bioassay diagnostic procedures to be positive for one of these virus diseases (Lightner et al., in press b).

A quarantined population in the enhancement procedure is reared under relatively crowded and stressful conditions. Postlarvae are best used for this test, which normally require 30 to 60 days. Random samples are taken at intervals throughout the test period, or nonrandom samples are selected as moribund animals are observed. Samples may be prepared for wet mount microscopic examination for BP and MBV, or preserved for histological evaluation. The enhancement procedure is far more sensitive than the direct sampling procedure for BP and MBV-caused diseases, and for IHHN disease in *P. stylirostris* (Lightner et al., in press b). Paynter et al. (in press) have found that diagnosis of HPV in captive wild *P. esculentus* in Australia may also lend itself to the enhancement procedure. Enhancement is not a suitable procedure for demonstration of IHHNV in asymptomatic carriers. For example, enhancement will not readily demonstrate IHHN to be present in Subadult or adult *P. stylirostris* that are IHHN epizootic survivors, or in species such as

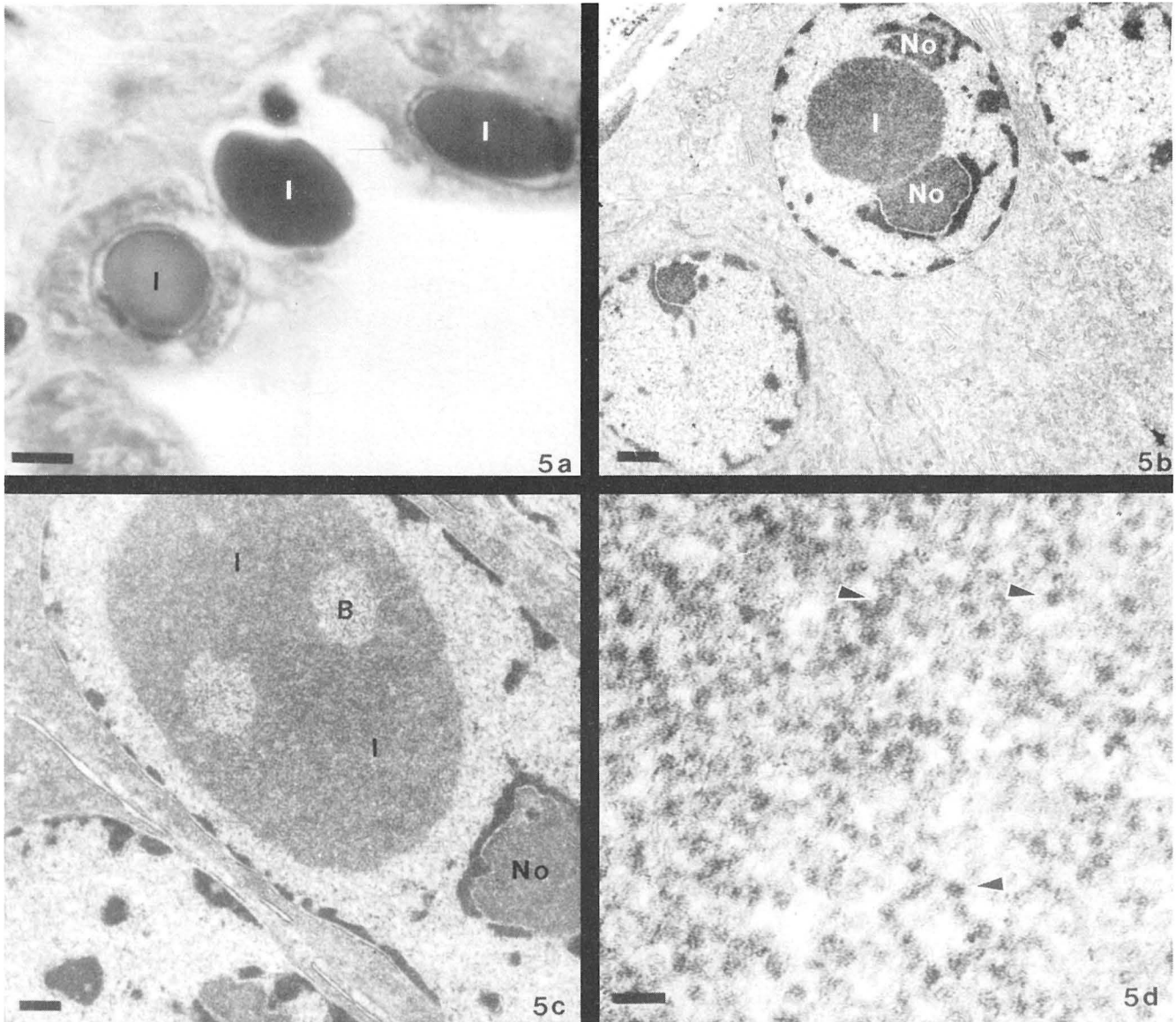
*P. vannamei* which are readily infected by the virus, but seldom show diagnosable infections (Lightner et al., 1983b).

Carriers of IHNV may be detected by bioassay with sensitive "indicator" shrimp. Indicator shrimp in this procedure known as IHNV-free (juvenile *P. stylirostris* of 0.05 to 4 g body weight) may be exposed to samples of suspect carrier shrimp by one or more of three methods: 1) injection with a cell-free filtrate prepared from a homogenate of sus-

pect carrier shrimp (the indicator shrimp will show signs of IHNV disease within 5 to 15 days if the suspect shrimp were infected with IHNV); 2) rearing in the same tank suspect carrier shrimp with indicator shrimp (the indicator shrimp will show signs of IHNV disease within 15 to 60 days); and 3) feeding chopped carcasses of suspect carrier shrimp to indicator shrimp (the indicator shrimp will show signs of IHNV within 15 to 60 days) (Lightner et al., 1983b, in press b).



**Fig. 4.** IHNV: 4a) Histological section of a typical IHNV intranuclear eosinophilic inclusion body (I) in a gill epidermal cell of a juvenile *P. stylirostris*,  $\times 1,100$ . Bar is  $5 \mu\text{m}$ ; 4b) TEM of an IHNV intranuclear inclusion body in a circulating hemocyte in the gills. Nuclear chromatin (C) has been displaced against the inner surface of the nuclear membrane, while the center of the nucleus has become filled with a proteinaceous granular (G) matrix that contains electron-dense spheres and strands (arrowheads),  $\times 15,000$ . Bar is  $0.5 \mu\text{m}$ ; 4c) Histological section of gills showing cells with eosinophilic IHNV intranuclear inclusions (I) and a basophilic cytoplasmic inclusion body (which is an IHNV virus paracrystalline array) in a gill epidermal cell,  $\times 1,520$ . Bar is  $5 \mu\text{m}$ ; 4d) TEM of a gill epidermal cell with masses of IHNV virus (V) and a paracrystalline array of virions (C) in the cytoplasm. The nucleus (N) contains no virions,  $\times 25,200$ . Bar is  $250 \text{ nm}$ ; 4e) Higher magnification TEM of IHNV virions in a paracrystalline array,  $\times 124,800$ . Bar is  $50 \text{ nm}$ ; 4f) Negative-stained purified preparation of 20 to 22 nm diameter IHNV virus from particles cesium chloride density gradient centrifugation (2% PTA),  $\times 410,000$ . Bar is  $20 \text{ nm}$ .



**Fig. 5.** Hepatopancreatic parvo-like virus (HPV): 5a) Histological section of a hepatopancreas tubule of *P. merguensis* that is heavily infected with HPV. Dense basophilic HPV inclusion bodies (I) are present within markedly hypertrophied host cell nuclei,  $\times 1,880$ . Bar is  $5 \mu\text{m}$  5b) TEM of an HPV-infected hepatopancreatocyte of *P. orientalis* that contains a developing intranuclear inclusion body (I). The inclusion body is composed of a granular virogenic stroma that is intimately associated with the host cell nucleoli (No),  $\times 6,120$ . Bar is  $1 \mu\text{m}$ ; 5c) TEM of a more advanced HPV inclusion body (I) which contains two intranuclear bodies (B) embedded in the virogenic stroma. The host cell nucleolus (No) has been displaced by the developing inclusion body,  $\times 12,600$ . Bar is  $0.5 \mu\text{m}$ ; 5d) A high magnification of the virogenic stroma of 5c in which masses of HPV virions are present. Profiles of some HPV particles are clearly angular (arrowheads),  $\times 147,000$ . Bar is  $50 \text{ nm}$ .

Actual diagnosis of infection by BP, MBV, BMN, HPV, and IHNV is dependent on microscopic or histologic demonstration of the particular cytopathology that is unique to each disease. Gross signs and behavior are usually not sufficiently specific in shrimp with infection by these penaeid viruses to be used reliably in diagnosing these diseases.

#### Bacterial and fungal diseases

**Bacteria.** A number of bacteria have been implicated as causes of disease and mortality in cultured penaeids, espe-

cially in the larval, postlarval and juvenile stages (Johnson, 1978; Lightner, 1983). Bacterial infections in shrimp may take three general forms: erosions of the cuticle covering the general body surface, gills, and appendages (bacterial necrosis and shell disease), localized lesions within the body, and generalized septicemias. Recent reports on the occurrence of bacterial diseases in cultured penaeids in Kuwait (Tareen, 1982), and China (Meng and Yu, 1980, 1982a, 1982b, 1983) are similar to previously reviewed reports from other shrimp culture groups (Lightner, 1977, 1983). While bacterial diseases of a probable primary bacterial etiology have been



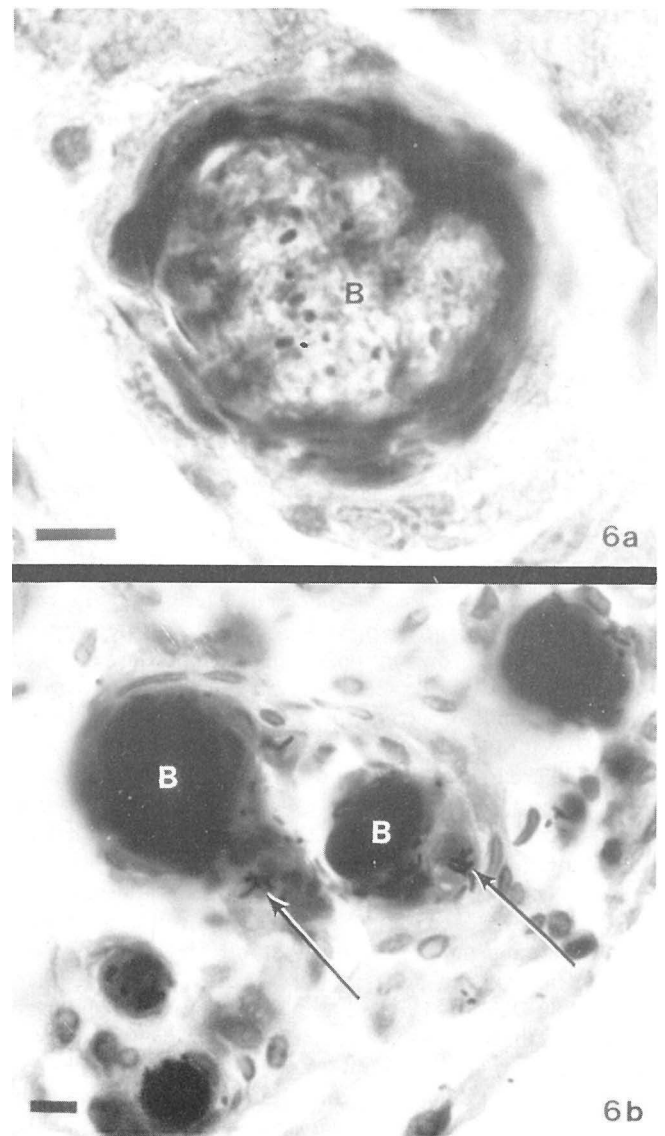
reported from penaeid shrimp (Nickelson and Vanderzant, 1971; Cook and Lofton, 1973), the majority are of a secondary etiology, occurring as a result of syndromes due to such things as ascorbic acid deficiency, toxins, wounds, extreme stress, etc. (Lightner, 1983). A number of reports in the literature support this observation. Many laboratory attempts have been made to complete Koch's postulates with bacterial isolates obtained from penaeids, and in each study a relatively massive inoculum had to be administered to overcome the natural defenses of the host and to produce disease and death in the experimental animals (Vanderzant et al., 1970; Lewis, 1973b; Lightner and Lewis, 1975; Corliss et al., 1977; Huang et al., 1981). One study showed that cell-free solutions of crude extracts of endotoxins and exotoxins of *Vibrio parahaemolyticus* and *V. alginolyticus* injected into *P. setiferus* produced significant mortalities with gross signs similar to those observed in actual bacterial infections (Leong and Hanrahan, 1980).

In every reported bacterial infection in penaeid shrimp reviewed up to 1983 (Lightner, 1983), motile, gram-negative, oxidase-positive, fermentative rods have been isolated from lesions or host hemolymph. Most isolates have been *Vibrio* spp., usually *V. alginolyticus*, *V. parahaemolyticus*, or *V. anguillarum*. Certain other gram-negative rods, including *Pseudomonas* spp., and *Aeromonas* spp. may occasionally be involved in bacterial disease syndromes in penaeid shrimp (Lightner, 1983). All of these genera and species have been reported to be among the normal microflora of penaeids (Vanderzant et al., 1970, 1971; Hood and Meyers, 1977; Yasuda and Kitao, 1980; Lewis et al., 1982). Although a variety of gram-positive cocci, including the etiological agent (*Aerococcus viridans*) that causes highly lethal Gaffkemia disease in *Homarus* lobsters, have been isolated from shrimp, none have been linked with disease in penaeids (Stewart and Rabin, 1970; Vanderzant et al., 1971; Vanderzant et al., 1972). Hence, it would appear that shrimp have only opportunistic pathogens that are part of their normal microflora. A possible exception to this was the discovery earlier this year of a gram-negative, acid-fast rod causing disease in adult *P. vannamei* (Lightner, unpub.). Shrimp infected with this microorganism were moribund when collected, but showed no externally apparent abnormalities. Histopathology, however, revealed that the acid-fast bacterium was present in very large numbers either encapsulated in melanized hemocyte nodules or in the tissues surrounding such granulomatous lesions in the host hepatopancreas, antennal gland, and mandibular organ (Fig. 6). The latter two organs were severely affected. Further studies on penaeid bacterial diseases should include tests for acid-fast microorganisms in the event that this pathogen has been overlooked in penaeids.

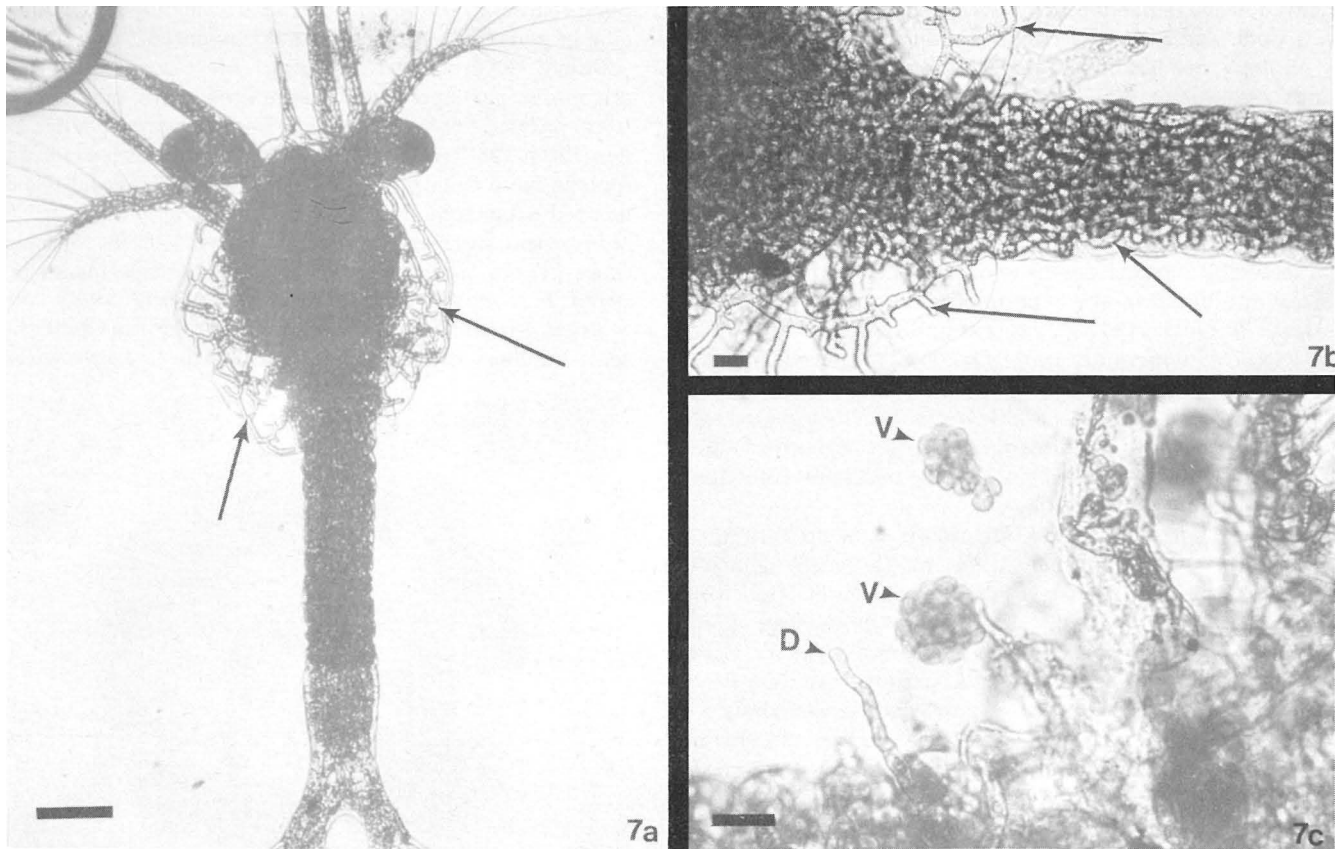
Several groups have reported effective therapy of these diseases using antibiotics such as Furanace, Furacin, Terramycin, Aureomycin, and Chloramphenicol, and antibacterial chemotherapeutics such as formalin, malachite green, and methylene blue (Aquacop, 1977; Tareen, 1982; Lightner, 1983). Vaccines against *Vibrio* sp. have been reported by Lewis and Lawrence (in press) to be potentially effective in preventing losses due to *Vibrio* spp. infections in aquarium and

pond-reared *P. setiferus*, but the efficacious use of this vaccine in penaeids remains to be documented.

**Fungi.** Several species of fungi infect penaeids, and some are major pathogens of these animals. No new species of fungi parasitic to penaeids have been recognized since Lightner (1981, 1983) reviewed the subject, although several more reports have been published recently that expand the documented geographic and host range of *Lagenidium* sp., *Sirolopidium* sp., and *Fusarium solani* (Figs. 7, 8, 9). Members of these genera were reported to cause disease losses in cultured *P. semisulcatus* in Kuwait (Tareen, 1982), and in *P. orientalis* cultured in the Yellow Sea region of China. Large-scale hatchery losses of eggs and larvae to *Lagenidium* sp.



**Fig. 6.** Acid-fast bacteria: Histological section of the mandibular organ of an adult *P. vannamei*. Present in the section are melanized hemocytic nodules that contain masses of gram-positive rod-shaped bacteria (B),  $\times 1,880$ . Bar is  $5\ \mu\text{m}$ ; 6b) The masses of acid-fast bacteria (B) and a few isolated rods (arrows) are acid-fast positive,  $\times 1,560$ . Bar is  $5\ \mu\text{m}$ . (Stains: 6a, Brown and Brenn histologic gram stain and 6b, Ziehl-Neelsen.)



**Fig. 7.** *Lagenidium callinectes*: 7a and 7b) A larval *P. setiferus* with an advanced infection of *Lagenidium*. Hyphae (arrows) nearly fill the body of the larva; 7c) Discharge tubes (D) with terminal vesicles (V) that contain maturing zoospores of *Lagenidium*. (Magnifications: 7a,  $\times 20$ . Bar is 0.5 mm; 7b,  $\times 44$ . Bar is  $100\mu\text{m}$ ; 7c,  $\times 78$ . Bar is  $100\mu\text{m}$ .)

and *Sirolopidium* sp. were reported (Meng and Yu, 1980, 1982a), and *Fusarium* sp. was reported to infect juvenile *P. orientalis* in grow-out ponds in the same area of China (Meng and Yu, 1982b, 1983). As was the case in most of the bacterial species reported from cultured penaeid shrimp, the imperfect fungus *Fusarium* sp. (all isolates that have been identified are *F. solani*) and the phycomycetous fungi *Lagenidium* sp. and *Sirolopidium* sp. appear to be present in virtually all shrimp culture facilities throughout the world. This is not surprising because each of the fungi has a wide host range or can exist as a free-living saprophyte (Johnson and Sparrow, 1961; Moss and Smith, 1984). While no effective chemotherapeutants have been found for treatment of *F. solani* infections in penaeids (Hatai et al., 1974; Lightner, 1981, 1983; Tareen, 1982), a number of effective chemotherapeutants have been identified and tested against *Lagenidium* sp. (Bland et al., 1976; Lio-Po et al., 1982).

The histopathology (Bian and Egusa, 1981) and pathogenesis (Hose et al., 1984) of *F. solani* infections in penaeids have been recently reported. Penaeids respond to invasion by *F. solani* hyphae with an intense hemocytic response that includes hemocyte encapsulation, melanization, and the deposition of collagen fibers within a granulomatous lesion (Fig. 9) that surrounds and isolates the invading hyphae (Bian and Egusa, 1981). Studies of these lesions by TEM, however, have shown that, despite the intensity of host res-

ponse, a large percentage of hemocyte-encapsulated *F. solani* hyphae remains viable within the granulomatous lesions (Fig. 9d; Lightner, 1981; Hose et al., 1984). Contributing to the pathogenesis of *F. solani*, in addition to its direct invasiveness and destructive effect on host tissues, are secondary bacterial infections and changes in the hemolymph content of the host. Hemolymph from severely *F. solani*-infected *P. californiensis* was hypoproteinemic, hemocytopenic, and frequently failed to coagulate (Hose et al., 1984).

#### Protozoan parasitic diseases

**Microsporidians.** Microsporidians (Protozoa, Microspora) cause a group of diseases in penaeids that are collectively called "cotton" or "milk shrimp disease." At least three genera of microsporidia, *Ameson* (= *Nosema*), *Agmasoma* (= *Thelohania*), and *Pleistophora*, are known to infect captive wild and cultured penaeids, especially in ponds or in enclosed natural bodies of water (Overstreet, 1982; Lightner, 1983). Tissues infected by these parasites include striated and smooth muscle, and the gonads. Infection prevalences in penaeid culture ponds have approached 10% (Couch, 1978). Severe infections in cultured penaeids may cause chronic disease mortality (Couch, 1978; Lightner, 1983), parasitic castration (Enriquez et al., 1980), as well as an unmarketable product.

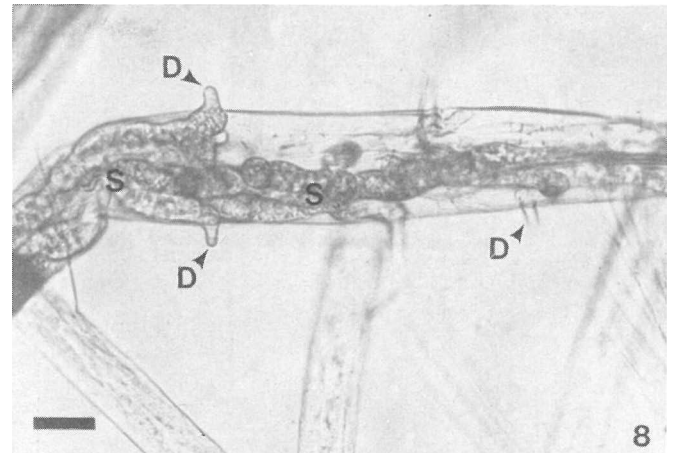
**Gregarines.** Gregarines (Protozoa, Apicomplexa) are common inhabitants of the guts of wild and pond-reared penaeids (Johnson, 1978; Overstreet, 1978; Couch, 1983). Two genera, *Nematopsis* and *Cephalolobus*, are known in penaeids (Lightner, 1983). These organisms use a mollusk for completion of their life cycle and, hence, may be excluded from tank and raceway culture systems (Johnson, 1978). Even when present in such large numbers as to occlude the midgut or hindgut lumen (Fig. 12), gregarines appear not to cause significant disease in penaeids.

**Noninfectious diseases**

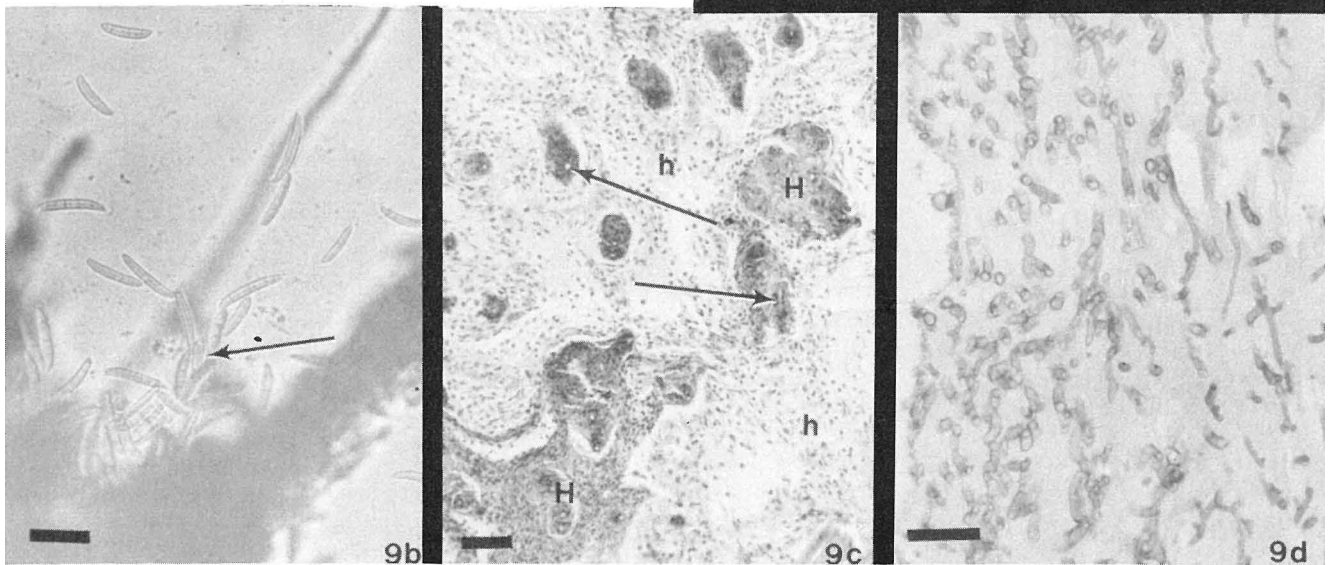
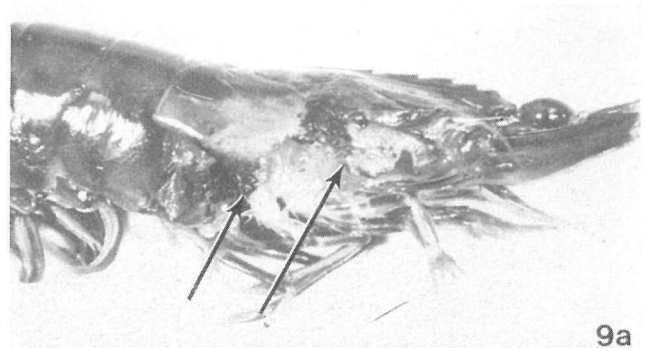
**Diseases caused by epicommissals**

Among the more serious diseases of cultured penaeids are those caused by noninfectious epicommissal organisms. These organisms are common and apparently ubiquitous in shrimp culture facilities. All life stages may be affected, but the most serious losses are encountered in juvenile and adult stages when the gills of the host become fouled (resulting in various forms of gill disease) by heavy infestations of epicommissal organisms such as filamentous bacteria, peritrich protozoans, and pinnate diatoms. Table 6 lists the more commonly observed and reported epicommissal organisms that alone, or with other epicommissals, cause "gill disease" and surface fouling in cultured penaeids. The more important diseases are discussed here.

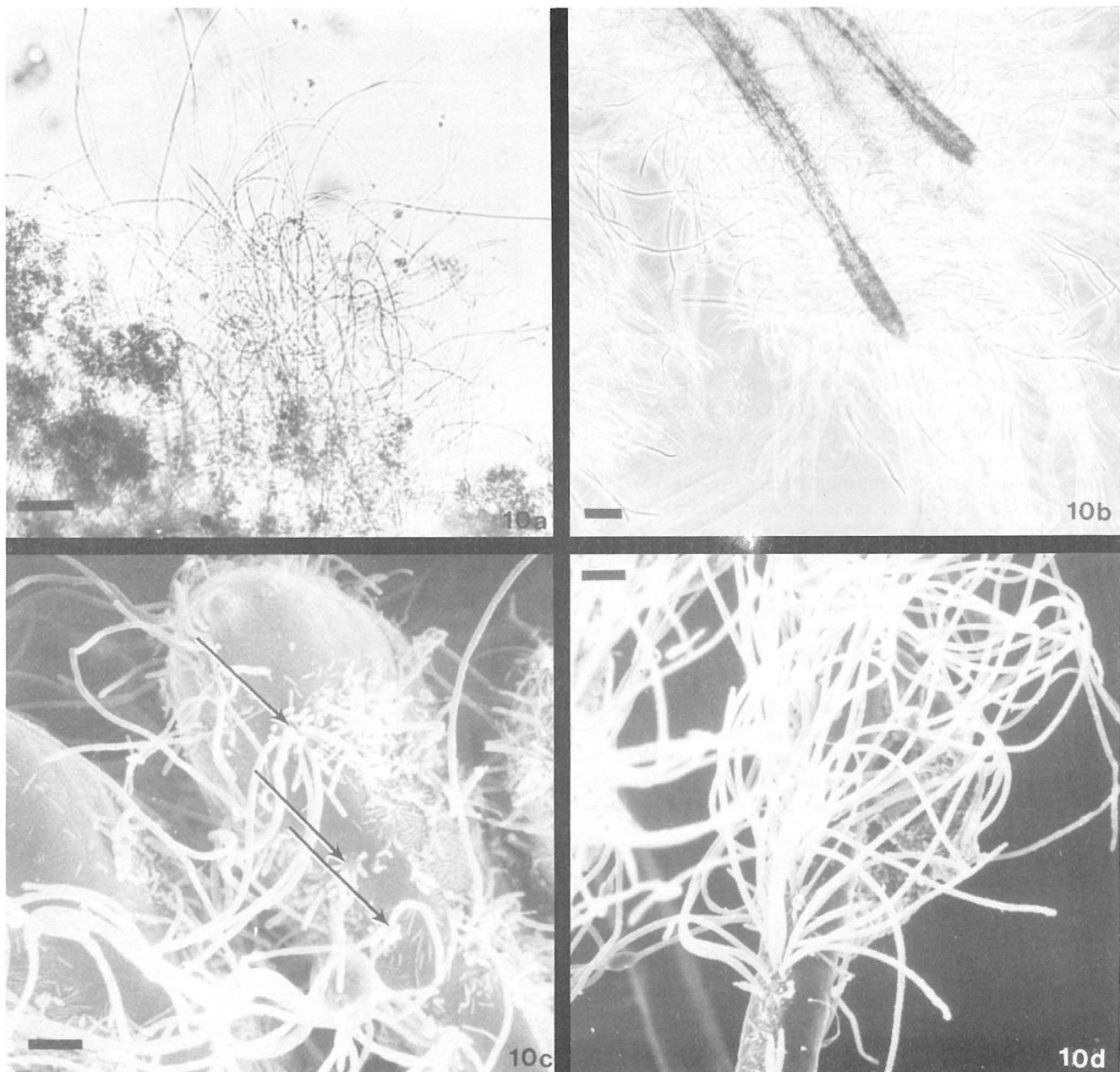
**Bacterial epicommissals.** *Leucothrix mucor* (Fig. 10) is a very common ubiquitous estuarine marine bacterium, reported from every penaeid culturing area of the world (McKee and Lightner, 1982; Lightner, 1983). Consistent with this are recent reports of losses due to *L. mucor* in penaeids



**Fig. 8.** *Sirolpidium* sp.: Wet mount of a larval *P. aztecus* infected with *Sirolpidium* sp. that shows sporangia (S) with short discharge tubes (D) that are developing from hyphae within an appendage,  $\times 160$ . Bar is  $50\mu\text{m}$ .



**Fig. 9.** *Fusarium solani*: 9a) *P. californiensis* with well-developed *Fusarium* lesions on the carapace and in the gills (arrows); 9b) Wet mount of conidiospores of *F. solani* from the gills of *P. californiensis*. Both canoe-shaped macroconidia and a microconidium (arrow) are present,  $\times 400$ . Bar is  $20\mu\text{m}$ ; 9c) Histologic section of a *Fusarium* lesion in the body wall and muscle of a *P. californiensis*. Masses of necrotic melanized hemocytes (H) and unmelanized hemocytes (h) encapsulate *Fusarium* hyphae (arrows) in the lesion which is invading the adjacent muscle (M),  $\times 132$ . Bar is  $50\mu\text{m}$ ; 9d) PAS-stained histological section of a *Fusarium* lesion showing the abundance of viable hyphae within a granulomatous lesion similar to that shown in 9c,  $\times 400$ . Bar is  $25\mu\text{m}$ .



**Fig. 10.** *Leucothrix mucor*: 10a) Surface fouling of gill lamellae of *P. aztecus* by the filamentous bacterium *L. mucor*,  $\times 180$ . Bar is  $50\mu\text{m}$ ; 10b) *L. mucor* on the tip of a gill mastigobranchia (functions to preen gills) of a *P. californiensis*,  $\times 120$ . Bar is  $50\mu\text{m}$ ; 10c) SEM of *Leucothrix* filaments on gill lamellae of a *P. stylirostris*. Note that many of the filaments arise from a basal rosette (arrows),  $\times 4,070$ . Bar is  $2\mu\text{m}$ ; 10d) SEM of *Leucothrix* filaments on the tip of a gill mastigobranchia of a *P. stylirostris*,  $\times 1,110$ . Bar is  $5\mu\text{m}$ .

cultured in Kuwait and China (Meng and Yu 1980, 1982b, 1983; Tareen 1982). *L. mucor* attaches to living and nonliving substrates, and in penaeid culture systems it readily attaches to the body surfaces of shrimp. In juvenile and older penaeids, *L. mucor* favors attachment to the gills and accessory gill structures (Fig. 10). Larval and postlarval penaeids may become so fouled by *L. mucor* filaments that respiration, feeding, locomotion, and molting may be seriously impaired, resulting in mortalities. *L. mucor* is noninvasive and it causes no demonstrable pathology to the surfaces to which it attaches (Lightner et al., 1975; Lightner, 1978a). Severity

of disease due to *L. mucor* in shrimp is related to organic loading of the culture system, to its oxygen content, and to the added stress of molting. Mortalities due to *L. mucor* surface and gill infestations are due to hypoxia.

Several other species of bacteria have been implicated in bacterial gill disease and surface fouling diseases of cultured penaeids (Table 6). Included among the filamentous forms are *Thiothrix* sp., *Cytophaga* sp. and *Flexibacteria* sp. (Lightner, 1983). Unlike *L. mucor*, inflammation and melanization of the gills often accompanies high levels of infestation by certain of these filamentous bacteria (Lightner, 1978a).

**Table 6.** Epicommensal organisms observed or reported to cause disease in cultured penaeids.

Bacteria	Ciliates (Protozoa)	Blue-Green Algae
<i>Leucothrix mucor</i>	<i>Zoothamnium</i> spp.	<i>Spirulina subsalsa</i>
<i>Thiothrix</i> sp.	<i>Epistylis</i> spp.	<i>Schizothrix calcicola</i>
<i>Flexibacter</i> sp.	<i>Vorticella</i> sp.	Diatoms
<i>Vibrio</i> spp.	<i>Lagenophrys</i> sp.	<i>Amphora</i> sp.
<i>Pseudomonas</i> spp.	Apostome ciliate	<i>Nitzschia</i> sp.
<i>Flavobacteria</i> sp.	Suctorians (Protozoa)	<i>Achanthes</i> sp.
<i>Aeromonas formicans</i>	<i>Acineta</i> spp.	

Lewis et al. (1982) reported aggregation of hatchery-reared *P. stylirostris* larvae by surface fouling due to infestations of *Pseudomonas piscicida*, *Aeromonas formicans*, and *Flavobacteria* sp.

**Protozoan epicommensals.** A number of species of protozoa have been reported to cause surface fouling and/or gill disease in all life stages of cultured penaeids (Table 6; Overstreet, 1982; Couch, 1983; Lightner, 1983). The most commonly reported protozoans include the peritrich ciliates (Fig. 11) *Epistylis* spp., *Zoothamnium* spp., and *Vorticella* spp.; the loricate Ciliate *Lagenophrys* sp.; an undescribed apostome Ciliate; and the suctorians *Acineta* spp. (Couch, 1978, 1983; Overstreet, 1978, 1982; Meng and Yu, 1980, 1983; Lightner, 1983). As was the case with bacterial epicommensals, these protozoans, when abundant on the body surfaces, appendages, or gills, can cause difficulties to the host in locomotion, feeding, molting, and respiration (Fig. 11). Like *L. mucor*, most of the protozoans cause no appreciable internal damage to the host surfaces or gills. The exception to this is the unidentified apostome Ciliate, which caused melanized hemocytic lesions in the gills of *P. aztecus* (Lightner, 1975; Overstreet, 1978, 1982).

**Algae.** A number of species of blue-green algae and diatoms (Table 6) have been reported to be among the epicommensal organisms causing surface fouling and gill disease in cultured and captive penaeids (Lightner, 1983). *Amphora* sp. has even been observed growing internally in the gills of *P. setiferus* reared in a shallow, nonturbid, well-lighted tank (Overstreet and Safford, 1980).

### Nutritional, toxic and environmental diseases

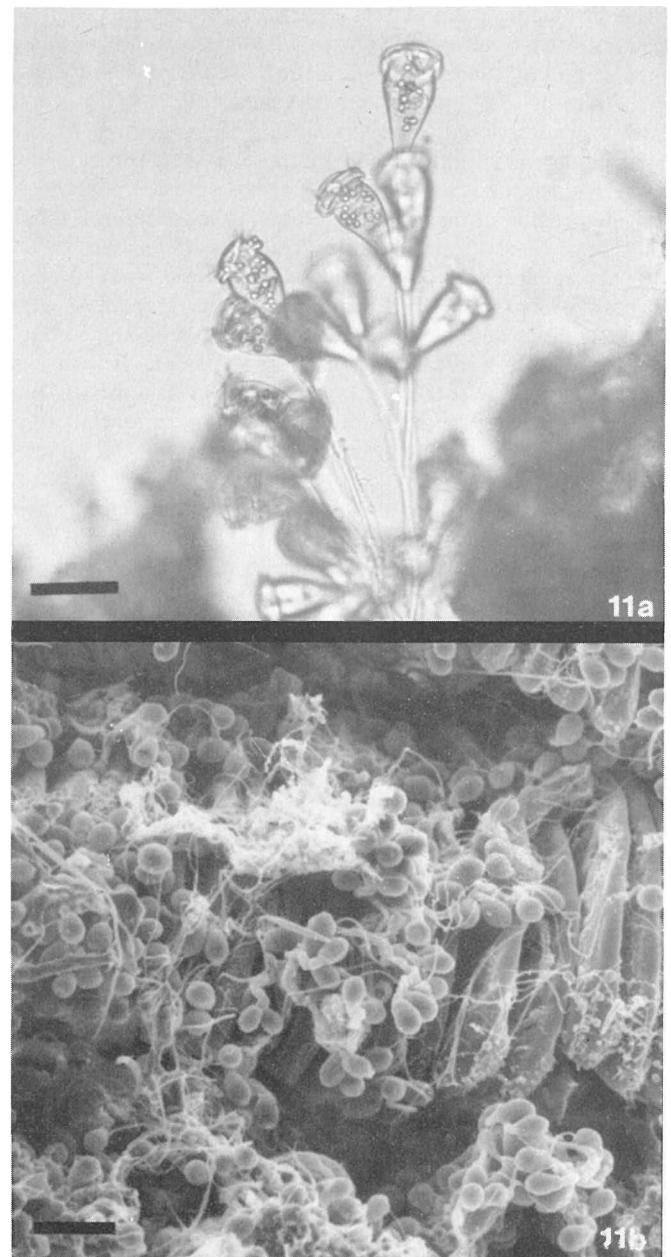
#### Nutritional diseases

Although a number of the nutritional requirements of cultured penaeids have been identified and such nutrients as the essential amino acids, cholesterol, linoleic acid,  $\beta$ -carotene and potassium are needed in penaeid diets for optimum growth, survival and appearance (New, 1976; Kanazawa, 1984), only one nutritional disease syndrome of cultured penaeids has been described in detail. That disease, called black death or shrimp scurvy (Lightner et al., 1977, 1979) occurs in penaeids which are reared in culture systems lacking algae and receiving diets with insufficient ascorbic acid (Fig. 13). The disease has not been observed in shrimp cultured in systems where there is at least some algae. Shrimp with black death possess melanized hemocytic lesions in the epithelial and supportive connective tissues of the general

body cuticle, the foregut and hindgut, the eyestalks, and the gills. The lesions are most prominent in tissues with a high collagen content (Hunter et al., 1979). Addition of L-ascorbic acid to the shrimp's ration or rearing shrimp in the presence of algae effectively prevents black death disease (Lightner et al., 1979).

#### Toxic diseases

Hemocytic enteritis (HE): Blooms of certain filamentous blue-green algae, all belonging to the family Oscillatoriaceae,



**Fig. 11.** *Zoothamnium* sp.: 11a) A colony of *Zoothamnium* from the gills of *P. californiensis*,  $\times 115$ . Bar is  $100\mu\text{m}$ ; 11b) SEM of gills of *P. stylirostris* that are heavily fouled by *Zoothamnium*,  $\times 220$ . Bar is  $50\mu\text{m}$ .

have been implicated as causing the disease syndrome HE in primarily young juvenile penaeids. The occurrence of HE seems to be ubiquitous, and examples of the disease exist from marine and freshwater shrimp aquaculture facilities in North America, Hawaii, Brazil, Philippines, and Israel (Table 7). One species of blue-green alga, shown experimentally to cause this syndrome, is *Schizothrix calcicola* (McKee, 1981). *S. calcicola* occurs in both fresh water and sea water, and has been reported to possess a potent endotoxin (Keleti et al., 1979). While HE is most commonly observed in early juvenile penaeids, it has been observed in Subadult penaeids as well.

The principal lesion of HE, which occurs as the result of algal endotoxin released in the gut from ingested algae, is a necrosis and marked hemocytic inflammation of the mucosal epithelium of the midgut and its caeca (Fig. 14; Lightner, 1978b), accompanied by necrosis and degeneration of the hepatopancreas (Lightner and Redman, 1984). The cause of death in shrimp with HE may be due to osmotic imbalances, poor absorption of nutrients, or to secondary bacterial infections. Species of *Vibrio*, usually *V. alginolyticus*, are the organisms most commonly isolated from the septic hemolymph of shrimp with HE (Lightner, 1983). Mortality rates in raceway-reared *P. stylirostris* with HE have reached 85% (Lightner, 1978b), but usually are less than 20%. Runting of shrimp affected with HE is a chronic effect in animals that survive the disease (Fig. 15) apparently due to midgut dysfunction and to the length of time required for the midgut mucosa to regenerate.

**Dinoflagellate poisoning.** Dinoflagellate blooms (red tides) have been circumstantially linked to serious mortalities of cultured penaeid shrimp in Mexico (Lightner et al., 1984), but a cause-and-effect relationship of mortality to the suspect species of dinoflagellates has not been experimentally demonstrated (Lightner, 1983). The occurrence of a toxicity syndrome called BSX, "Blue Shrimp Syndrome Unknown" in *P. californiensis* and *P. stylirostris* cultured in Mexico (Lightner, 1983) has been correlated with the occurrence of red tides. Shrimp with BSX die during molting or following handling stress, and in an affected population, a large percentage of the shrimp has been observed to develop "blunt heads" (Fig. 23). This condition was thought to develop from

**Table 7.** Geographic locations and species of cultured shrimps and prawns in which hemocytic enteritis has been observed.

Species	Geographic location	Reference
<i>Penaeus duorarum</i>	Florida	Nimmo et al., 1977
<i>P. stylirostris</i> <i>P. vannamei</i> , and <i>P. californiensis</i>	Mexico	Lightner et al., 1978; Lightner, 1983
<i>P. vannamei</i> , <i>P. stylirostris</i> , and <i>P. japonicus</i>	Hawaii	Lightner (unpub.)
<i>P. monodon</i>	Philippines	Lightner (unpub.)
<i>P. stylirostris</i>	Israel	Lightner (unpub.)
<i>Macrobrachium</i> <i>rosenbergii</i>	Hawaii, Philippines and Brazil	Brock, 1983; Lightner (unpub.)

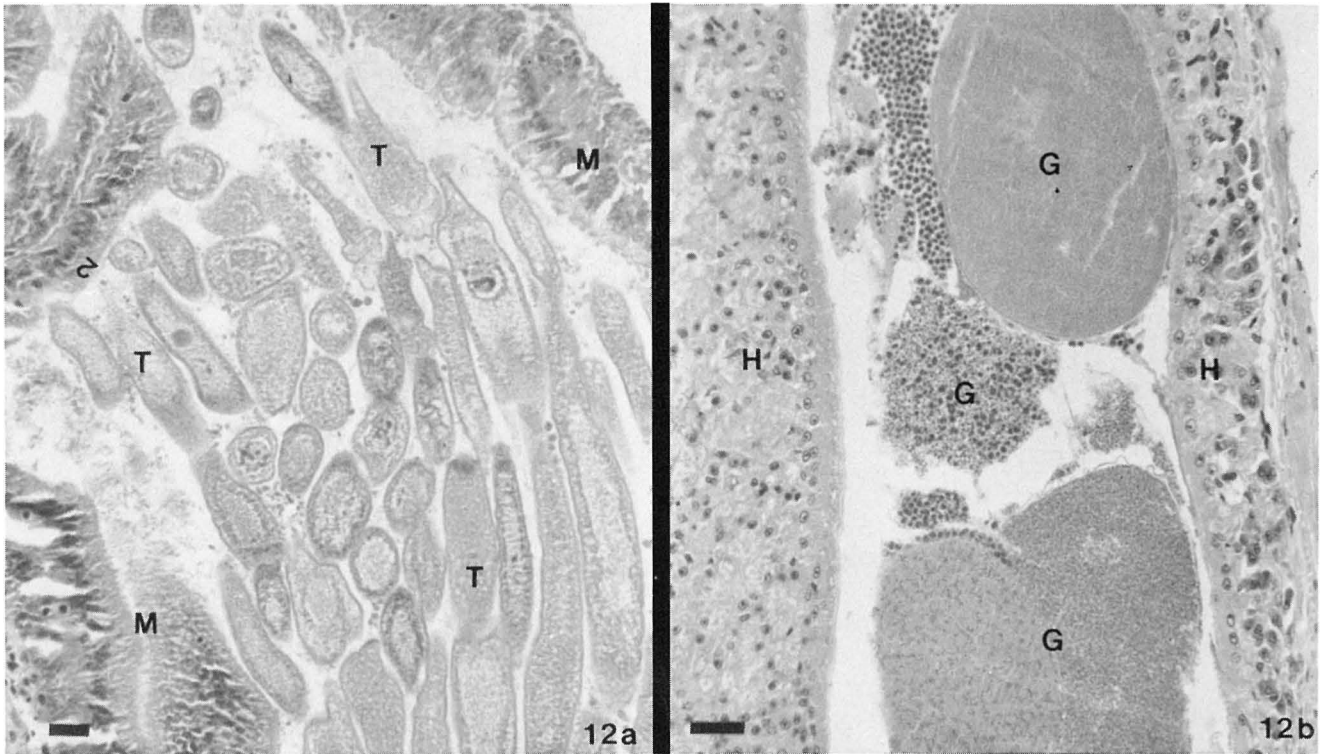
damage to the head appendages from the convulsive behavior pattern that occurs in this syndrome (Lightner, 1983). Dinoflagellate toxins are thought to be nontoxic to crustaceans (Sievers, 1969), but only short-term toxicity tests have been run on shrimp. However, during those tests, the few shrimp that molted also died. That observation and the circumstantial association of red tides and the BSX syndrome in Mexico indicate that the importance of red tide toxins to penaeids may be significant.

**Aflatoxicosis and red disease.** Aflatoxicosis and red disease are discussed together here because of the close similarity of their histopathology (Lightner et al., 1982; Lightner and Redman, in press b). However, the etiology of red disease is unknown, and while it may have a toxic cause, the possible role of an infectious agent in its etiology has not been completely explored. Both of these diseases have as their principal feature a necrosis of the hepatopancreas that is accompanied by marked intertubular hemocytic inflammation, tubule encapsulation, and melanization (Figs. 16, 17).

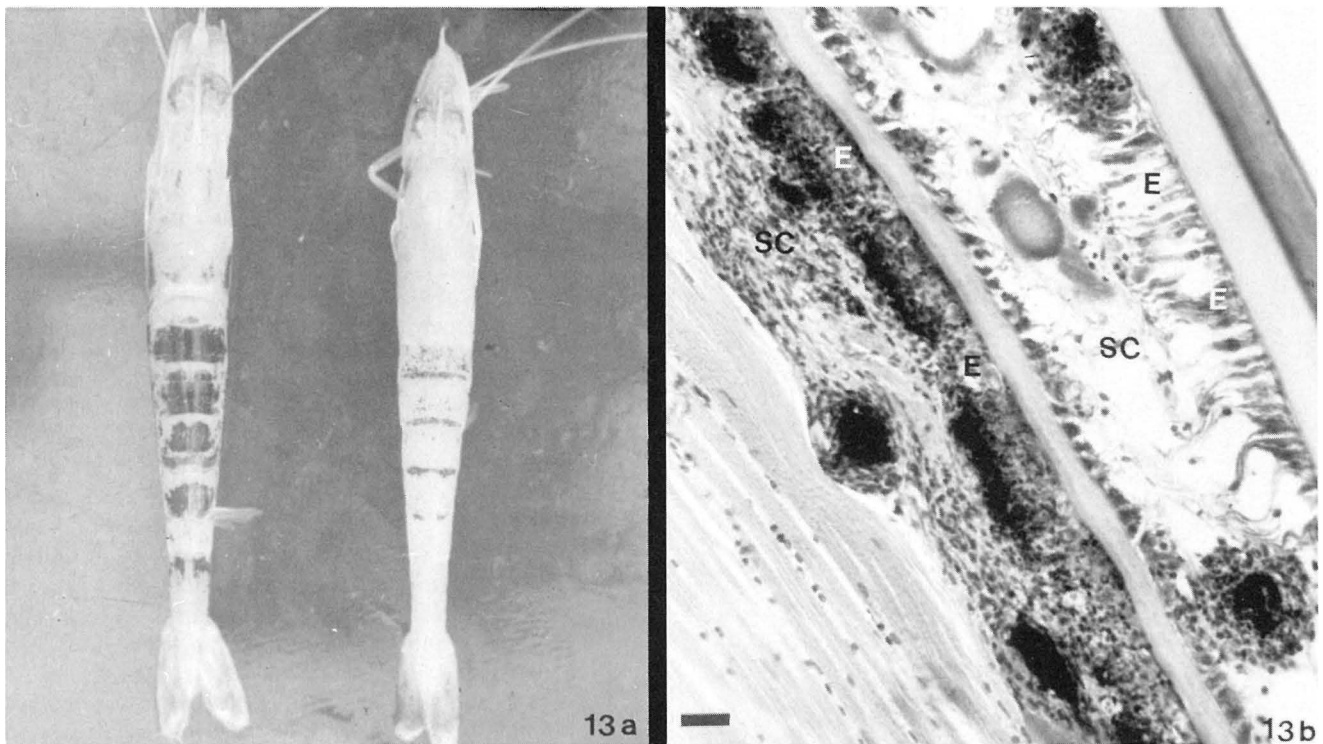
**Aflatoxicosis.** Necrosis and inflammation of the hepatopancreas, mandibular organ, and hematopoietic organs are the principal features of artificially induced aflatoxicosis (Fig. 16; Lightner et al., 1982). Although aflatoxicosis has not been proven to be an important disease of cultured penaeids, the mechanism for its being an important disease is in place. Penaeids reared in semi-intensive or intensive systems are fed artificial diets that may contain ingredients, which on occasion, can contain aflatoxin in sufficient amounts to result in aflatoxicosis (Arafa et al., 1979; Wiseman et al., 1982). Aflatoxin could also be produced "in situ" in penaeid feeds improperly stored under warm and humid conditions typical of penaeid culture regions (Wiseman et al., 1982).

The principal lesions of aflatoxicosis in penaeids (Lightner et al., 1982) occur in the hepatopancreas and the mandibular organ. In the hepatopancreas, acute and subacute aflatoxicosis is expressed as necrosis of the hepatopancreatic tubule epithelium that proceeds from the proximal portion of the tubules to the peripheral tubule tips (Fig. 16). A marked intertubular hemocytic inflammation followed by encapsulation and fibrosis of affected tubules follows in subacute and chronic aflatoxicosis, but does not develop in acute aflatoxicosis. The mandibular organ in aflatoxicosis displays a necrosis of the peripheral epithelial cells of cords within the gland that progresses proximally to the central vein (Fig. 16). Only a slight hemocytic inflammation accompanies the degenerative changes in the mandibular organ (Lightner et al., 1982).

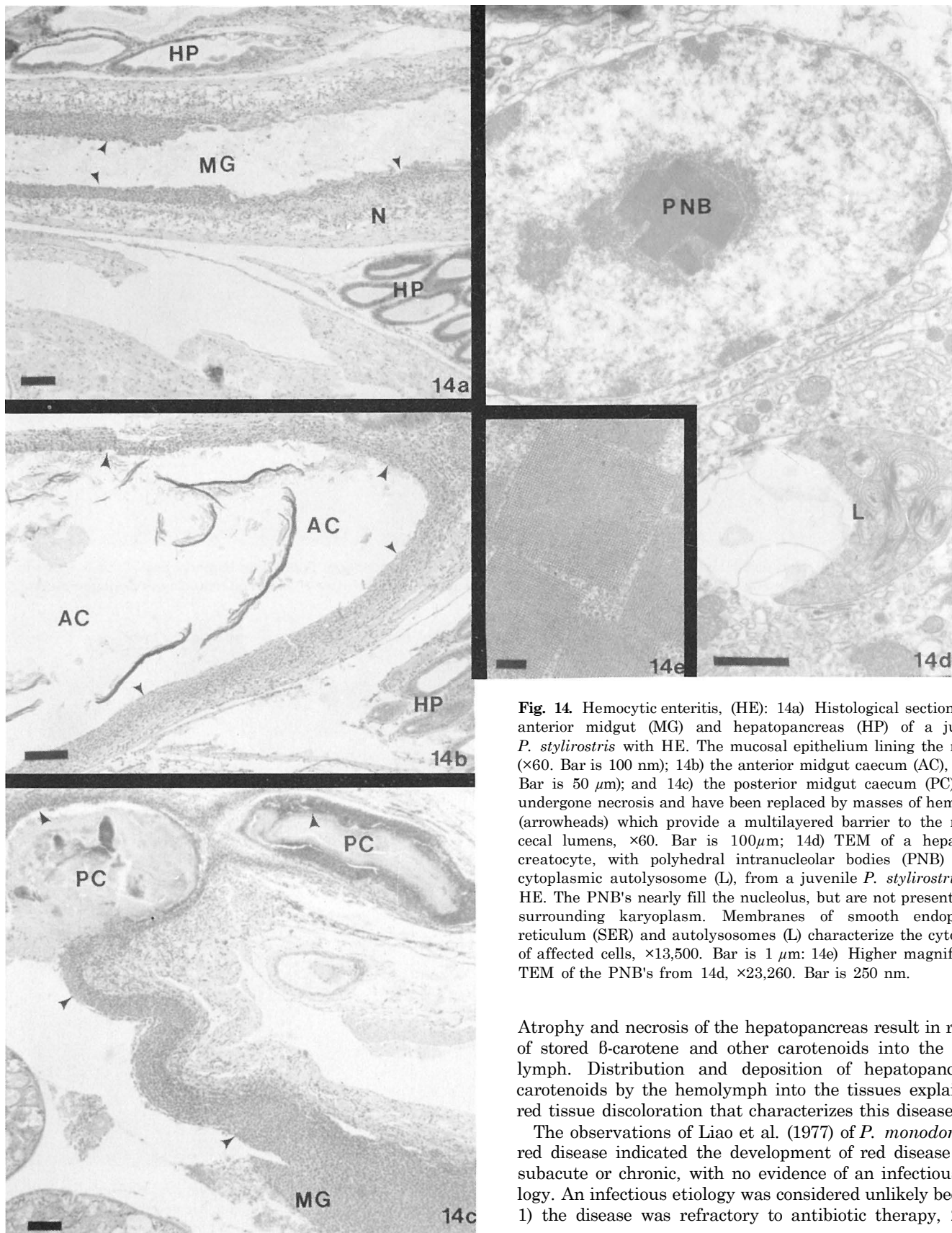
**Red disease.** Red discoloration or red disease was first noted in Taiwan (Liao, 1977; Liao et al., 1977) in cultured *P. monodon*. The disease has also been observed in captive wild adult *P. monodon* and in juvenile and adult cultured *P. monodon* in the Philippines (J.F. LeBitoux and C. Emerson, pers. comm., 1982) and in pond-reared *P. stylirostris* in Hawaii (Lightner and Redman, in press b). Liao (1977) noted that red disease in some years in Taiwan was "quite serious" especially in cultured adult *P. monodon*. The hepatopancreas of normal decapod crustaceans contains a variety of carotenoid pigments, with most of the total body content of  $\beta$ -carotene being stored in the hepatopancreas (Goodwin, 1960).



**Fig. 12.** Gregarines: 12a) Histological section of the anterior midgut (M) of a *P. monodon*. Numerous trophozoites (T) of a cephaline gregarine nearly fill the gut lumen,  $\times 52$ . Bar is  $100\ \mu\text{m}$ ; 12b) Section of the hindgut of the same *P. monodon* showing several gametocysts (G) in a crypt of the hindgut lumen (H),  $\times 265$ . Bar is  $25\ \mu\text{m}$ .



**Fig. 13.** Ascorbic acid deficiency syndrome ("black death disease"): 13a) Juvenile *P. californiensis* showing melanized subcuticular lesions that are typical of black death disease; 13b) Histological section through a cuticular lesion. Melanized hemocytic nodules and granulomas are present in the cuticular epidermis (E) and subcutis (SC) at this site where the cuticle of two abdominal segments overlap,  $\times 265$ . Bar is  $25\ \mu\text{m}$ .

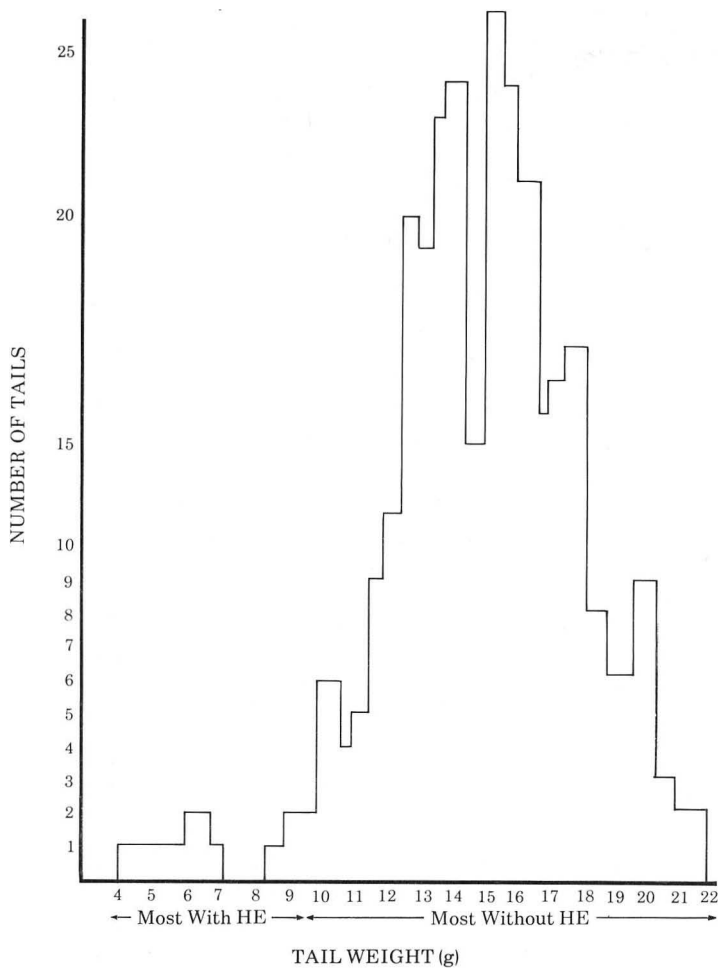


**Fig. 14.** Hemocytic enteritis, (HE): 14a) Histological section of the anterior midgut (MG) and hepatopancreas (HP) of a juvenile *P. stylirostris* with HE. The mucosal epithelium lining the midgut ( $\times 60$ . Bar is 100  $\mu\text{m}$ ); 14b) the anterior midgut caecum (AC), ( $\times 150$ . Bar is 50  $\mu\text{m}$ ); and 14c) the posterior midgut caecum (PC), have undergone necrosis and have been replaced by masses of hemocytes (arrowheads) which provide a multilayered barrier to the midgut cecal lumens,  $\times 60$ . Bar is 100  $\mu\text{m}$ ; 14d) TEM of a hepatopancreatocyte, with polyhedral intranucleolar bodies (PNB) and a cytoplasmic autolysosome (L), from a juvenile *P. stylirostris* with HE. The PNB's nearly fill the nucleolus, but are not present in the surrounding karyoplasm. Membranes of smooth endoplasmic reticulum (SER) and autolysosomes (L) characterize the cytoplasm of affected cells,  $\times 13,500$ . Bar is 1  $\mu\text{m}$ ; 14e) Higher magnification TEM of the PNB's from 14d,  $\times 23,260$ . Bar is 250 nm.

Atrophy and necrosis of the hepatopancreas result in release of stored  $\beta$ -carotene and other carotenoids into the hemolymph. Distribution and deposition of hepatopancreatic carotenoids by the hemolymph into the tissues explain the red tissue discoloration that characterizes this disease.

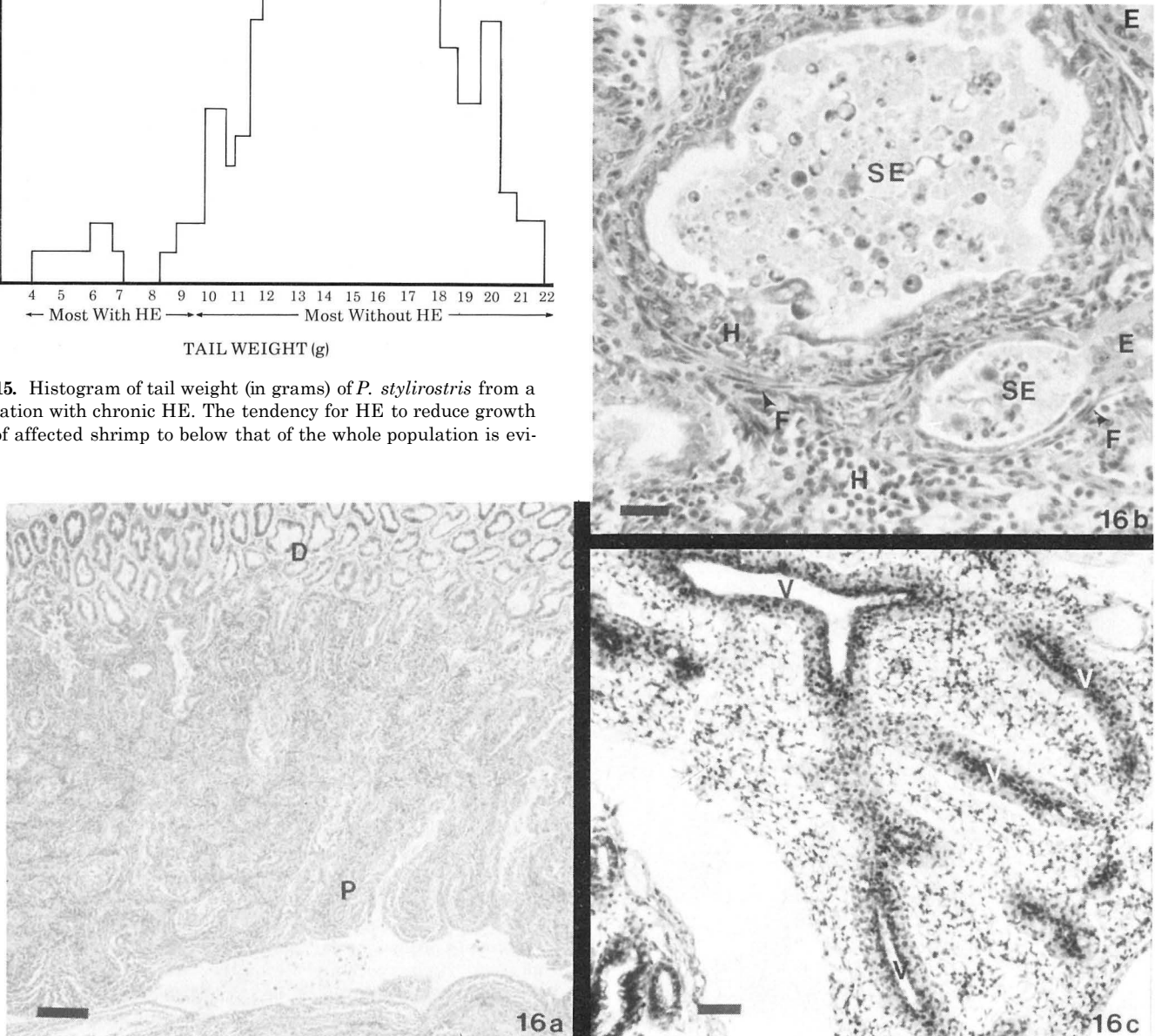
The observations of Liao et al. (1977) of *P. monodon* with red disease indicated the development of red disease to be subacute or chronic, with no evidence of an infectious etiology. An infectious etiology was considered unlikely because: 1) the disease was refractory to antibiotic therapy, 2) the

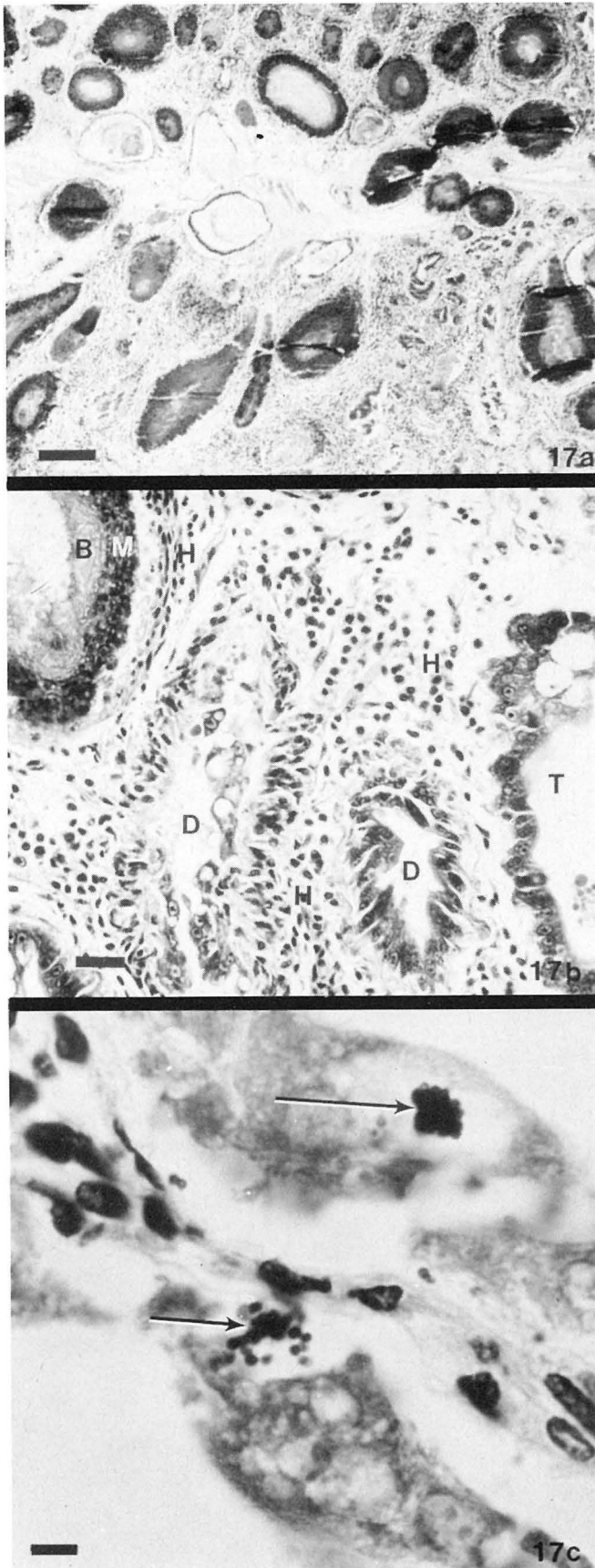




**Fig. 15.** Histogram of tail weight (in grams) of *P. stylirostris* from a population with chronic HE. The tendency for HE to reduce growth rate of affected shrimp to below that of the whole population is evident.

**Fig. 16.** Aflatoxicosis: 16a) Histological section of the hepatopancreas of a juvenile *P. stylirostris* with subacute experimentally induced aflatoxicosis. Degeneration and necrosis of the organ has proceeded distally (D, at top) from the proximal (P, at bottom) portion of the organ's tubules. The distal tubule tips are only slightly affected, while the medial and proximal portions of the tubules have been destroyed,  $\times 50$ . Bar is  $150 \mu\text{m}$ ; 16b) Higher magnification of the medial portion of several hepatopancreatic tubules undergoing necrosis. Degeneration is proceeding from proximal (left) to distal (right). The tubule epithelium in the proximal region has been sloughed (SE) into the tubule lumen, while the more distal epithelium (E) remains attached. An intense intertubular inflammatory response consisting of hemocytes (H) and fibroblasts (F) is present surrounding the affected tubules,  $\times 285$ . Bar is  $25 \mu\text{m}$ ; 16c) Histological section of the mandibular organ of a *P. stylirostris* with subacute aflatoxicosis. Normal epithelial cells surround the central vessels (V), but peripheral cells show extensive vacuolization, diminution of cytoplasm, and pyknotic nuclei,  $\times 130$ . Bar is  $50 \mu\text{m}$ .



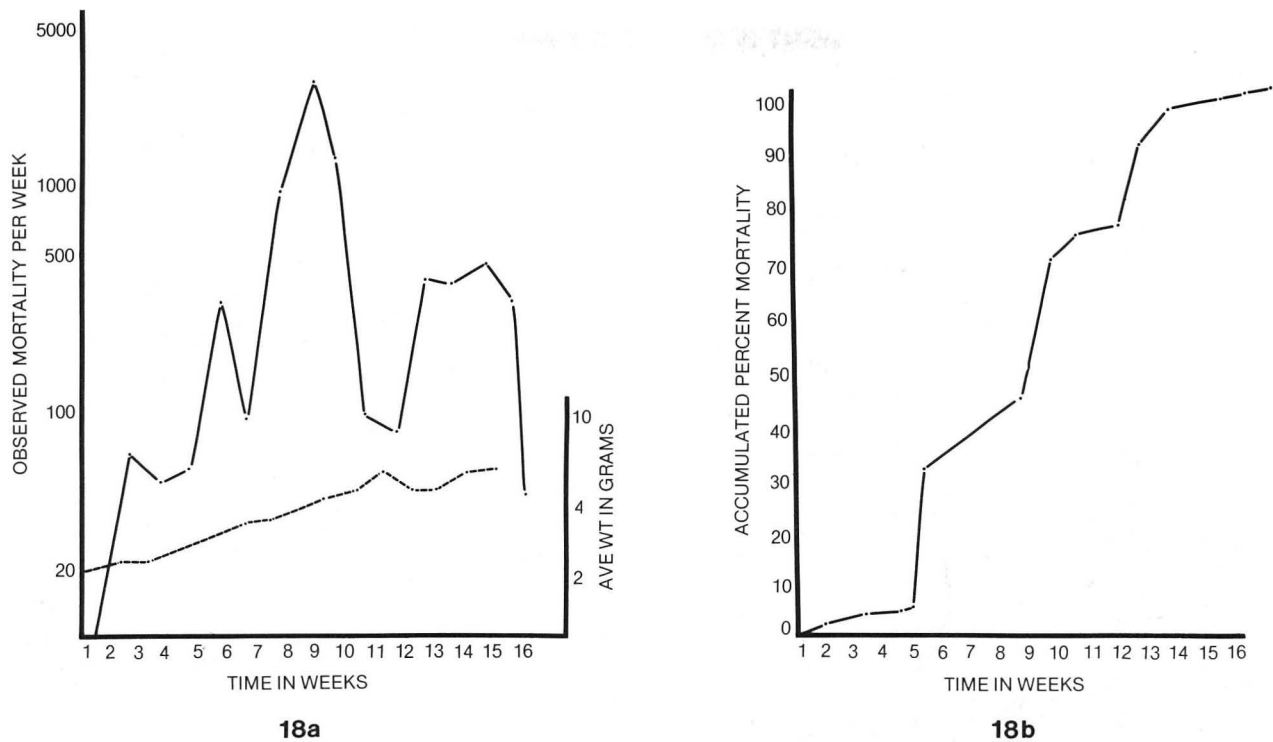


disease was observed only in *P. monodon*, even when polycultured with *P. penicillatus* and *P. semisulcatus*, and 3) because attempts failed to transmit the disease to unaffected *P. monodon*. Liao et al. (1977) suggested a link between feeding rancid fish and red disease, because the disease was not observed when care was taken to insure that only fresh fish were fed. However, C. Emerson (pers. comm.) noted that red disease in the Philippines occurred in pond-reared and captive wild *P. monodon* fed exclusively artificial diets. Emerson indicated, however, that in his experience red disease was most common in manure-fertilized ponds with thick anaerobic detritus deposits.

Liao et al. (1977) described the sequential development of red disease in *P. monodon*: Affected shrimp passed through four stages, with the earliest detectable signs of the disease being a yellowish-green discoloration of the shrimp body. Otherwise shrimp so affected remained active and displayed normal behavior. During the next two to four days affected shrimp became reddish, with the normally white gills and the pleopods also becoming reddish. Finally after five to seven days, affected shrimp became distinctly red and totally lost their normal brown and tan pigment (banded) pattern. Shrimp in the final stages of the disease were lethargic, anorectic and showed a tendency to excessive surface fouling by epicomensal organisms. The amount of body fluid in the cephalothorax increased over normal shrimp and had a foul odor. The hepatopancreas was reported to be yellow or "pale."

Histological examination of *P. monodon* and *P. stylirostris* with red disease revealed a marked atrophy of the hepatopancreas and the presence of numerous melanized inflammatory lesions in the hepatopancreas, antennal gland, mandibular organ, gonads, midgut and gills (Lightner and Redman, in press). Hepatopancreatic inflammatory lesions were the most consistently observed lesion type (Fig. 17). Affected hepatopancreata were atrophied (reduced by as much as 50% of expected normal size), usually contained multiple hemocyte-encapsulated hepatopancreas tubules with necrotic or sloughed epithelial linings, and possessed a marked hemocytic infiltration in the intertubular spaces (Fig. 17). Brown and Brenn gram-staining of affected hepatopancreata

**Fig. 17.** Red disease: 17a) Histological section of the hepatopancreas of a juvenile *P. monodon* with red disease. Most hepatopancreatic tubules are heavily encapsulated with hemocytes, melanized and contain masses of necrotic tissue debris and bacteria. Only a few normally appearing hepatopancreatic tubules (T, in upper right) are present in this section,  $\times 50$ . Bar is  $150 \mu\text{m}$  17b) A higher magnification photomicrograph of a portion of the same section shown in 17a. Several stages of hepatopancreatic tubule degeneration are shown. A nearly normal tubule (T), with its brush-bordered simple columnar lining epithelium, is separated by two degenerating tubules (D) from the remnants of another hemocyte-encapsulated (H) and melanized hemocyte-lined tubule (M) that has lost its lining epithelium and now contains a mass of tissue debris and bacteria (B),  $\times 265$ . Bar is  $25 \mu\text{m}$ ; 17c) Gram-positive cocci are commonly present in the hepatopancreas of *P. monodon* with red disease. These cocci are seen either free in the lumen of the hepatopancreas tubules or in cytoplasmic vacuoles (arrows) of tubule epithelial cells (Brown and Brenn tissue gram stain),  $\times 1,320$ . Bar is  $5 \mu\text{m}$ .



**Fig. 18.** Gut and nerve syndrome (GNS): 18a) Plots of mortality and growth rate in a population of raceway-cultured juvenile *P. japonicus* with GNS; 18b) Plot of accumulated percent mortality due to GNS in same population as shown in 18a.

of *P. monodon* and *P. stylirostris* showed the tubule lesions to contain masses of gram-negative rod-shaped bacteria and in *P. monodon* occasional prominent clusters of large (1.0 to 1.5  $\mu\text{m}$  diameter) gram-positive cocci or short rods (Fig. 17c). Unlike the gram-negative rods that were always present in tissue debris in the lumen of hemocyte-encapsulated tubules, the gram-positive organisms in *P. monodon* were observed in clusters within cytoplasmic vacuoles of tubule epithelial cells, as well as in the lumen debris.

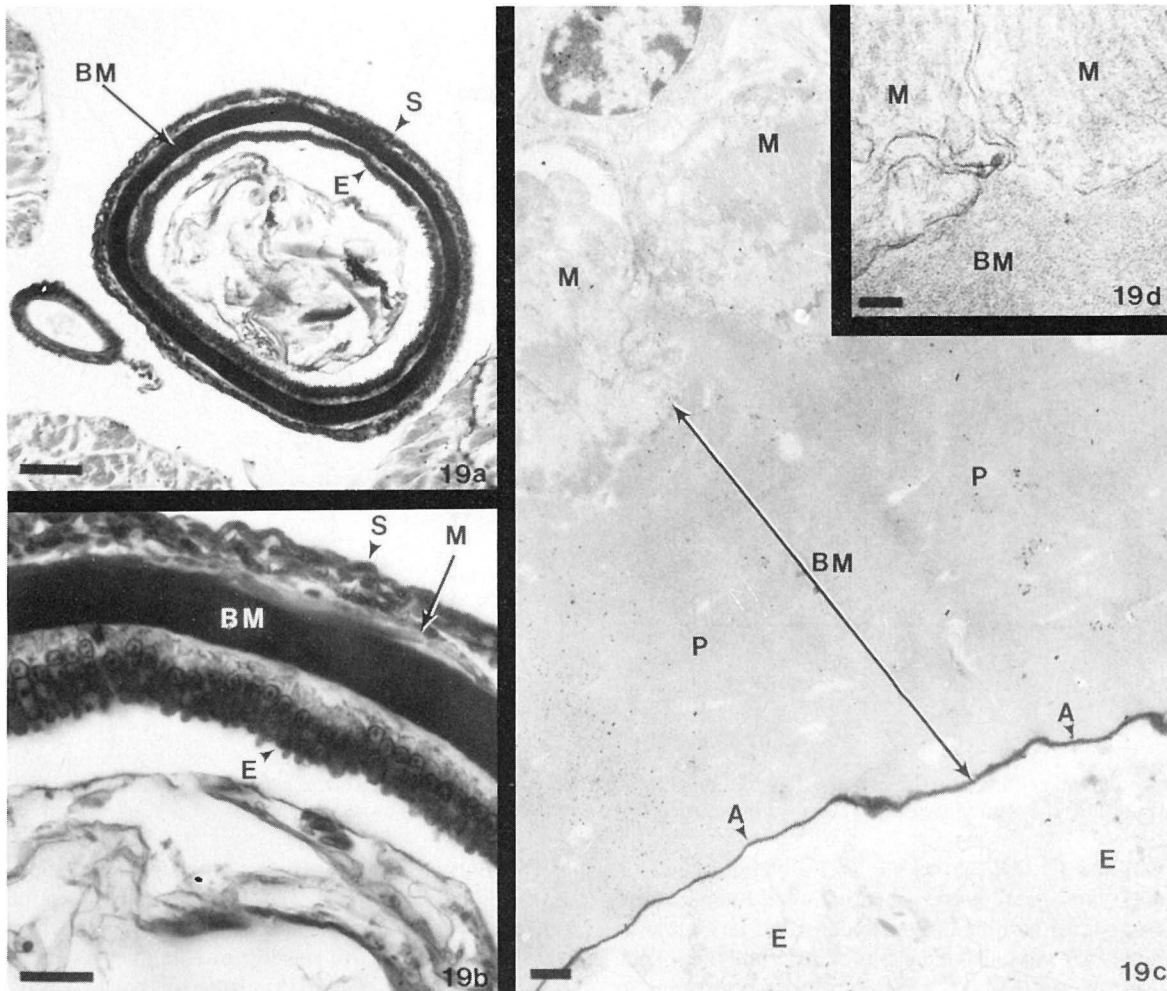
The significance of the relatively large numbers of gram-positive bacteria present in many of the *P. monodon* hepatopancreata with red disease is not known. Gram-positive bacteria do not typically make up a significant part of the normal microflora of penaeid shrimps and prawns (Vanderzant et al., 1970; Vanderzant et al., 1972; Lewis, 1973a; Yasuda and Kitao, 1980) or the known pathogens of penaeids (Lightner, 1983). The absence of these gram-positive bacteria in the Hawaii-reared *P. stylirostris* with red disease suggests either that they are not the etiological agent of red disease or that red disease may be a generalized syndrome, with more than one cause, resulting from a necrosis of the hepatopancreas and release of its content of carotenoid pigments into the hemolymph (Lightner and Redman, in press b).

**Gut and nerve syndrome.** This idiopathic proliferation condition affecting the midgut and ventral nerve cord has only been observed in populations of postlarval and juvenile *P. japonicus* reared in ponds, tanks, and raceways in Hawaii (Lightner et al., 1984). It has apparently not been observed in *P. japonicus* reared in Japan or elsewhere. The disease was named gut and nerve syndrome (GNS) to reflect its idio-

pathic nature and the principal organs affected. The severity and high prevalence of GNS in virtually all populations of cultured *P. japonicus* studied since 1980 in Hawaii had precluded the successful rearing of this species in Hawaii, particularly in high density culture (Fig. 18; Lightner et al., 1984). Although there is no evidence to support the hypothesis, GNS is hypothesized to be caused by a toxin, possibly an algal toxin, that is unique to Hawaii (Lightner et al., 1984). The principal lesions observed in *P. japonicus* with GNS are a hypertrophy of the anterior midgut mucosal epithelium basement membrane (BM) and a hyperplasia of the epineurium that covers the ventral nerve cord and segmental ganglia in the gnathothorax (Figs. 19, 20). There seemed to be a positive correlation between increased thickness of the BM and disease (i.e. poor growth, anorexia, extreme surface fouling, abdominal muscle necrosis, and opportunistic bacterial and fungal, usually *F. solani*, infections), and a possible relationship between ataxia, lethargy, and reduced escape response and the degree of hyperplasia of the epineurium (Lightner et al., 1984).

**Black gill disease.** A number of disease syndromes of cultured penaeids are accompanied by the presence of black (melanized) inflamed lesions in the gills (Fig. 21; Lightner, 1977; Lightner and Redman, 1977). In fact, black gills may accompany many of the syndromes described earlier in this review (Table 8), and are also frequently a sign of toxic syndromes caused by chemical irritants including certain heavy metals, oil, ammonia and nitrite, and ozone.

**Gas-bubble disease.** Gas-bubble disease has been reported to occur in penaeid shrimp as a result of supersaturation of



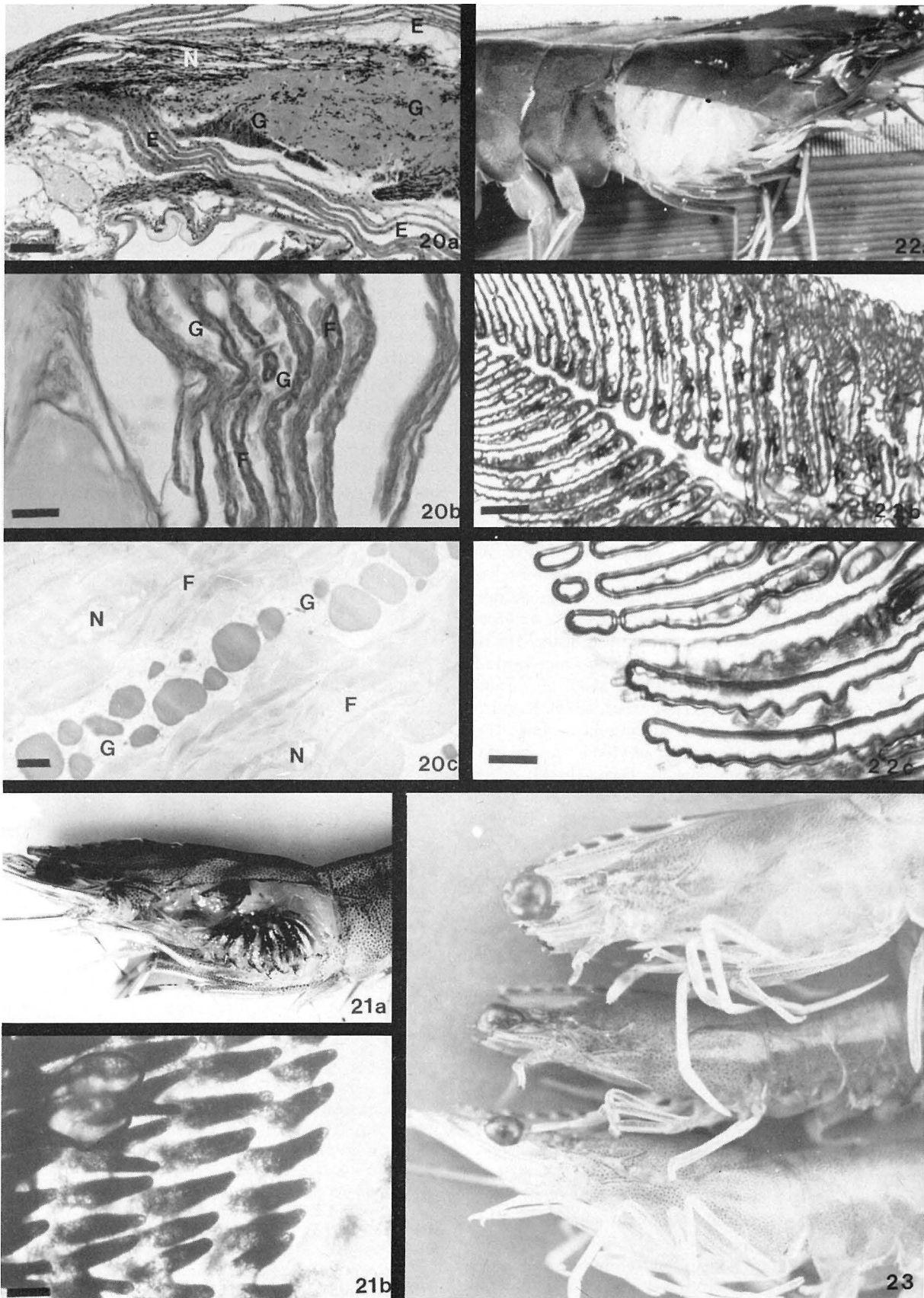
**Fig. 19.** Gut and nerve syndrome (GNS): 19a and 19b) Cross-section of the midgut from a *P. japonicus* with GNS. The mucosal epithelium (E) rests on a hypertrophied basement membrane (BM). The circular and longitudinal muscle layers (M) and the serosa (S) are normal in appearance (PAS staining) (19a,  $\times 180$ . Bar is  $50\ \mu\text{m}$ ; 19b,  $\times 530$ . Bar is  $20\ \mu\text{m}$ ); 19c) TEM of a hypertrophied basement membrane (BM). The mucosal epithelium (E) surface of the BM shows a slightly hypertrophied apical layer (A) that is more electron dense than the greatly hypertrophied proximal layer (P), which lies adjacent to smooth muscle cells (M),  $\times 6,000$ . Bar is  $1\ \mu\text{m}$ ; 19d) A higher magnification TEM of the proximal portion of hypertrophied BM that shows it to be composed of fine fibrils embedded in a finely granular matrix,  $\times 65,000$ . Bar is  $100\ \text{nm}$ .

**Fig. 20.** Gut and nerve syndrome (GNS): 20a) A sagittal section of the ventral nerve cord (N) and a segmental ganglion (G) in the gnathothorax of a juvenile *P. japonicus* with GNS. The epineurium (E) is composed of multiple repeating layers, rather than the normal single layers,  $\times 86$ . Bar is  $100\ \mu\text{m}$ ; 20b) A higher magnification of a multi-layered epineurium covering the ventral nerve cord is shown with seven repeating PAS-positive fibrous bands (F) that alternate with layers of granulocytes (G) that contain prominent PAS-negative granules,  $\times 340$ . Bar is  $25\ \mu\text{m}$ ; 20c) TEM of two fibrous layers (F) separated by a granulocytic layer (G). The nuclei of fibrocytes (N) are present in the fibrous layers, which are composed of bundles of collagen fibers arranged at oblique angles,  $\times 3,300$ . Bar is  $2\ \mu\text{m}$ .

**Fig. 21.** Black gills: 21a) A juvenile *P. stylirostris* with black gills due to an intense hemocytic inflammatory response to damaged or necrotic gill tissues. Melanization of hemocytes and surrounding tissues results in the black color; 21b) Wet mount of a gill process from a juvenile *P. californiensis* with severe gill melanization,  $\times 32$ . Bar is  $250\ \mu\text{m}$ .

**Fig. 22.** Gas-bubble disease: 22a) A juvenile *P. stylirostris* with gas-bubble disease. The gills of this shrimp appear white due to numerous gas bubbles within the gill lamellae; 22b and 22c) Wet mount of a gill process from a *P. stylirostris* with gas-bubble disease. At low magnification (22b,  $\times 36$ . Bar is  $250\ \mu\text{m}$ ), hemocoel rami in the gill process are outlined by gas emboli that, at a higher magnification (22c,  $\times 86$ . Bar is  $100\ \mu\text{m}$ ), are shown to block all hemolymph circulation, thereby stopping respiration.

**Fig. 23.** Dinoflagellate toxicity syndrome: Juvenile *P. stylirostris* with gross signs of the disease syndrome BSX that has been circumstantially linked to red-tide toxins. Blunting of the head (of top two, bottom shrimp is normal) is due to erosion of the antennae, antennules, rostrum, antennal blades, and portions of the eyes.



**Table 8.** Biological and chemical agents reported to cause black gills in penaeid shrimp.

Agent	Reference
Virus	
IHHNV	Lightner et al. (in press)
Bacteria	
<i>Flexibacter</i> sp.	Lightner, 1978a
<i>Cytophaga</i> sp.	
<i>Vibrio (Beneckea)</i> spp.	Cook and Lofton, 1973; Cipriani et al., 1980
Fungi	
<i>Fusarium solani</i>	Egusa and Ueda, 1972
Protozoa	
Apostome Ciliate	Couch, 1978; Overstreet, 1978
Nutritional Deficiency	
Black Death (shrimp scurvy)	Lightner et al., 1979
Chemical	
Cadmium, copper	Couch, 1978
Potassium permanganate	Lightner, 1977
Ozone	Danald et al., 1979
Ammonia and nitrite	Lightner, 1983

atmospheric gases and oxygen (Lightner, 1983). Shrimp are similar to fish in their sensitivity to supersaturation of atmospheric gases. Although the level of nitrogen or atmospheric gas supersaturation required to cause gas-bubble disease in penaeids has not been formally studied, a threshold of about 118% saturation is assumed (Lightner, 1983). Oxygen-caused gas-bubble disease in penaeids was reported to occur when oxygen reached or exceeded 250% of normal saturation in seawater (Supplee and Lightner, 1976). Regardless of the gas causing gas-bubble disease in shrimp, the clinical signs are the same. The most obvious sign of gas-bubble disease is that shrimp with it, float. (In all other diseases, dead or dying shrimp sink.) Examination of fresh preparations of gills or whole tissue by microscopy reveals the presence of gas bubbles (Fig. 22).

Cramped tail. This occasionally observed condition of penaeid shrimp has been reported to occur in the summer months, when both air and water temperatures are high (Johnson, 1975b; Lightner, 1977; Liao et al., 1977; Meng and Yu, 1980). Penaeids with cramped tails (while still alive) have a dorsal flexure of the abdomen that cannot be straightened. The condition typically follows handling, although shrimp have been observed with cramped tails in undisturbed ponds (Johnson, 1975b). The cause of cramped tail is unknown, but its occurrence only during summer suggests that elevated water and air temperatures, the handling of shrimp in air that is warmer than the culture system water, and other stresses may contribute to the cause of the condition.

Muscle necrosis (spontaneous necrosis). Muscle necrosis is the name given to a condition in all penaeid species that is characterized by whitish opaque areas in the striated musculature, especially in the distal abdominal segments (Rigdon and Baxter, 1970). The condition follows periods of severe stress (from low oxygen, sudden temperature or salinity changes, severe gill fouling, etc.) (Lakshmi et al., 1978; Lightner, 1983). It is reversible in its initial stages, but it

may be lethal if large areas are affected. "Tail rot" is the name given to the chronic and usually septic form of the disease when the distal portion of the abdomen (or appendages) becomes necrotic, turns red, and begins to slough.

### Summary

There are five areas of research that should receive emphasis in the next several years in penaeid disease research: 1) Appropriately equipped laboratories in each of the major penaeid culture areas should identify and catalog those diseases occurring in wild populations and in culture facilities in their region; 2) Penaeid diagnostic laboratories should use or strive to develop for general use "standardized" diagnostic procedures whenever possible, especially for highly infectious agents such as the penaeid viruses; 3) Penaeid cell culture methods for primary cultures or cell lines must be developed to aid in the development of a much needed rapid, sensitive diagnostic test or tests for the penaeid viruses; 4) Improved methods of disease prevention, control, or chemotherapy are needed for many of the penaeid diseases now adversely affecting the penaeid culture industry; and 5) Approval is needed from those government regulatory agencies (such as the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency) for drugs and chemicals used as pesticides and chemotherapeutics in penaeid culture that may pose a health risk to humans.

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