

Tissue Specificities of *Thelohania duorara*, *Agmasoma penaei*, and *Pleistophora* sp., Microsporidian Parasites of Pink Shrimp, *Penaeus duorarum*

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The pathology of pink shrimp, *Penaeus duorarum*, infected with the microsporidians *Thelohania duorara*, *Agmasoma penaei*, and *Pleistophora* sp. was described. Infections of *T. duorara* were widespread in most tissues; spores were located throughout the hemocoel, at the periphery of all striated muscle bundles, and in muscle and connective tissue surrounding the digestive tract. *A. penaei* infections invaded only dorsal abdominal muscles, muscles adjacent to blood vessels, and ovaries. Infected muscles and ovaries were eventually completely destroyed. Masses of *A. penaei* spores were often engulfed by hemocytes. *Pleistophora* sp. infected the interior of all striated muscles. Infected muscles were never completely destroyed but were often atrophied.

KEY WORDS: *Agmasoma penaei*; *Pleistophora* sp.; *Thelohania duorara*; *Penaeus duorarum*; Microsporidia; tissue specificity; pink shrimp.

INTRODUCTION

Among the most obvious and most harmful diseases of Crustacea are those caused by microsporidia. The effect of microsporidian infection on natural populations of Crustacea has been most commonly reported for crayfish. The microsporidian *Thelohania contejeani* has been directly implicated in large reductions of populations of *Astacus astacus* in the Federal Republic of Germany and in Finland (Sumari, 1970). Microsporidian infection has also been proposed as a possible contributory factor to vast mortalities of crayfish, *Cambarellus puer* and *C. shufeldti*, in Louisiana (Sogandares-Bernal, 1962).

As in the case with most marine organisms, reports of mass mortalities in marine Crustacea are few. Sindermann (1970) noted the difficulties in obtaining such information and concluded that mass mortalities are much more common than are indicated by scientific reports. One of the few reports of mass mortality in shrimp was made by Viosca (1945), who described

a previously unpublished report of an epizootic in penaeid shrimp in the Gulf of Mexico; in 1929, 90% of the white shrimp, *Penaeus setiferus*, along the Louisiana coast were infected by what appears to be the microsporidian *Agmasoma penaei*. An unknown microsporidian was also reported to infect up to 89.5% of white shrimp in South Carolina (Miglarese and Shealy, 1974).

In this study three microsporidians known to be responsible for the "cotton" or "milk" condition of penaeid shrimp were examined histologically with the light microscope. These Microsporida appear to belong to the genera *Thelohania*, *Agmasoma*, and *Pleistophora*. *Thelohania duorara* was originally described with light microscopy in pink shrimp, *Penaeus duorarum*, by Iversen and Manning (1959). Additional electron microscopical studies (Kelly, 1975) of this pathogen have been conducted. Sprague (1950) provided the first light microscopical study of *A. penaei* in white shrimp. Hazard and Oldacre (1975) later examined the ultrastructure of this species. On the basis of its octospore size and shape, polar filament length and diameter, and tissue specificity, the microsporid-

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ian in pink shrimp is tentatively identified as *A. penaei*. More definitive studies are necessary to determine if *A. penaei* does in fact infect both white and pink shrimp.

Comparisons of the *Pleistophora* sp. from pink shrimp with that described in brown shrimp, *Penaeus aztecus*, (see Rigdon and Baxter, 1970; Contransitch, 1970) are difficult without ultrastructural descriptions; however, similarities do exist in the size of spores and pansporoblasts, and in pathology. Electron microscopical studies are needed to determine whether these pathogens in pink and brown shrimp are indeed the same species.

MATERIALS AND METHODS

Juvenile and adult pink shrimp, *Penaeus duorarum*, 55–130 mm total length, showing gross symptoms of microsporidiosis were selected from bait shrimp catches made in southern Biscayne Bay over a 13-month period. Shrimp were sacrificed by immersion in Carnoy's fixative followed by injection of fixative through the exoskeleton with a hypodermic syringe. Immediately after death, shrimp were cut into 1-cm cubes. Before embedding shrimp pieces in Paraplast (Aloe Scientific Co.) the exoskeleton was removed. Eight-micrometer sections were stained with DelafIELD hematoxylin and eosin or with Masson trichrome stain.

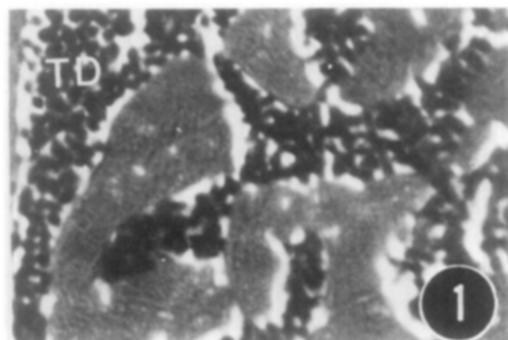
RESULTS

Thelohania duorara: Gross Pathologic Manifestations

Pink shrimp infected with *T. duorara* exhibited the typical "cotton" or "milk" condition, the entire abdomen and thorax being white. Muscle had a soft texture. Lightly infected specimens were not observed.

Thelohania duorara: Tissues Infected

Muscle. While gross examinations indicated that muscle tissue itself was the site of *T. duorara* infection, histological evidence showed that spores and pansporoblasts were located within the interstices and at the outer surfaces of whole muscles and muscle fascicles (Fig. 1). Dense concentra-



FIGS. 1–9. All figures are 8- μm sections of pink shrimp, *Penaeus duorarum*.

FIG. 1. *Thelohania duorara* spores at outer surface and within the interstices of abdominal muscle fascicles. $\times 540$.

tions of *T. duorara* spores and pansporoblasts in these areas made it difficult to ascertain whether superficial muscle fibers had actually been lysed or were merely concealed by the spores. All striated muscles in all of the *T. duorara*-infected specimens examined in this study exhibited this same pattern of infection. In no case was the complete destruction of individual, whole muscles observed.

Digestive gland. Developing sporoblasts were often visible within the large vacuoles of digestive gland secretory cells. Although free spores and pansporoblasts were usually heavily concentrated within the hemocoel adjacent to digestive gland diverticula, digestive gland tissue remained intact and free of mature spores.

Hemocyte-forming organ. Pockets of *T. duorara* spores were often present in the interstitial region of the hemocyte-forming organ. The heaviest concentration of *T. duorara* spores and sporoblasts occurred in the hemocoel, ventral to this organ (Fig. 2).

Digestive tract. *T. duorara* spores and sporoblasts were in discrete masses within the connective tissue surrounding the foregut.

Connective tissue and muscle lining the midgut contained heavy concentrations of spores. The epithelium of the midgut appeared normal.

Connective tissue and muscle lining the hindgut contained numerous spores and

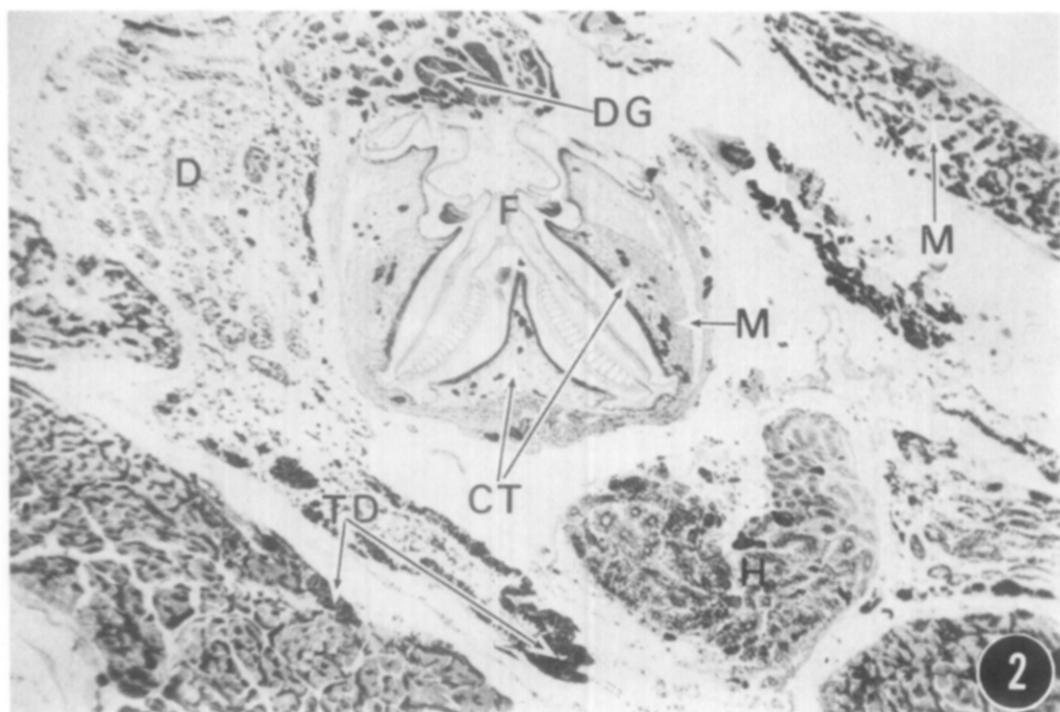


FIG. 2. Cross section of thorax showing *Thelohania duorara* infection in thoracic muscles, in connective tissue and muscle lining the foregut, adjacent to the dorsal gland and digestive gland, and within the hemocyte-forming organ. CT, connective tissue; D, digestive gland; DG, dorsal gland; F, foregut; H, hemocyte-forming organ; M, muscle; TD, *Thelohania duorara* spores. $\times 45$.

sporoblasts. Gland-like tissue in the hindgut appeared normal (Fig. 3).

Agmasoma penaei: Gross Pathologic Manifestation

Pink shrimp infected with *A. penaei* were characterized by distinctive, opaque patches along the dorsal midline, varying in length from 1 cm to the entire length of the abdomen and thorax.

Agmasoma penaei: Tissues Infected

Muscle. *A. penaei* showed marked specificity in the pattern of muscle infection. Infections were almost totally restricted to the dorsolateral and dorsomedial abdominal muscles. Superficial dorsal abdominal muscles were uninfected, as were most other muscles ventral to the midgut. Infected muscles rarely adjoined other infected muscles (Fig. 4).

When infections did occur in ventral abdominal areas they were restricted to mus-

cles in the following areas: (1) adjacent to intersegmental blood vessels (Fig. 5), (2) adjoining the exoskeleton, and (3) surrounding blood vessels within periopods. Infection of the massive anterior oblique muscles was never observed.

A. penaei infected both superficial and internal muscle fibers of a muscle bundle. In advanced stages of infection, entire muscles were destroyed. In the latter case the perimysium and epimysium continued to surround spore masses and allowed them to retain the shape of the muscle tissue that had been present.

Both light- and dark-stained masses of *A. penaei* spores were observed in spaces formerly occupied by abdominal muscles. Hemocytes encapsulated light-stained spore masses. The more common dark-stained masses of spores lacked hemocyte capsules (Fig. 6).

Thorax, digestive gland. Within the



FIG. 3. Cross section of hindgut region. Note *Thelohania duorara* spores in surrounding muscles and within the connective tissue and muscle of hindgut and hindgut gland. HG, hindgut; HL, hindgut gland; TD, *Thelohania duorara* spores. $\times 60$.

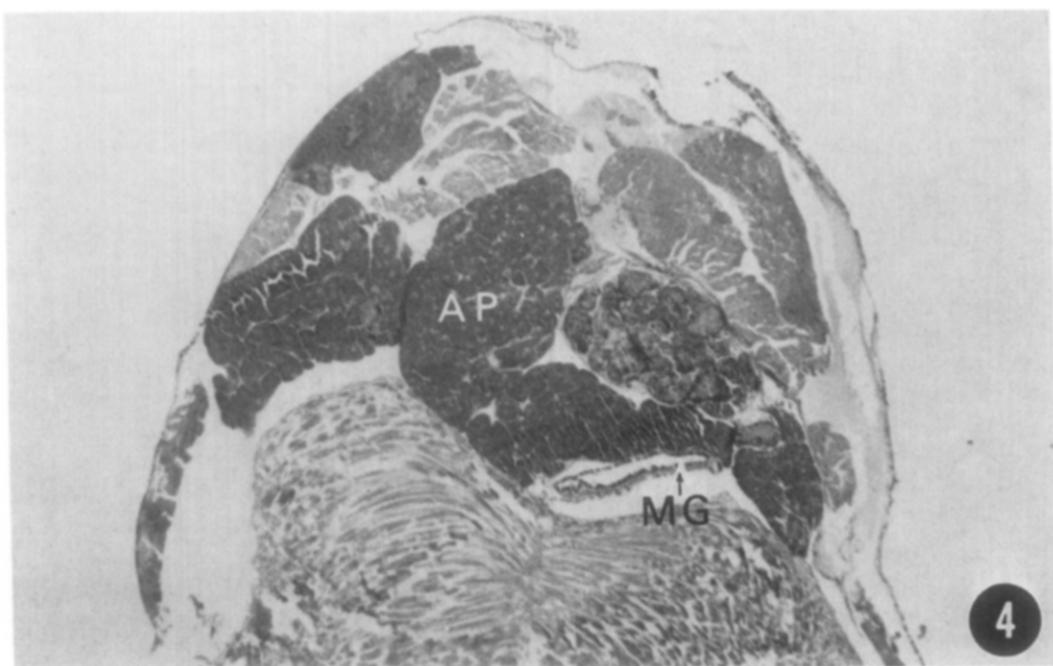


FIG. 4. Cross section of *Agmasoma penaei*-infected abdomen. Spores have replaced the dorsolateral and dorsomedial abdominal muscles. AP, *Agmasoma penaei* spores; MG, midgut. $\times 30$.

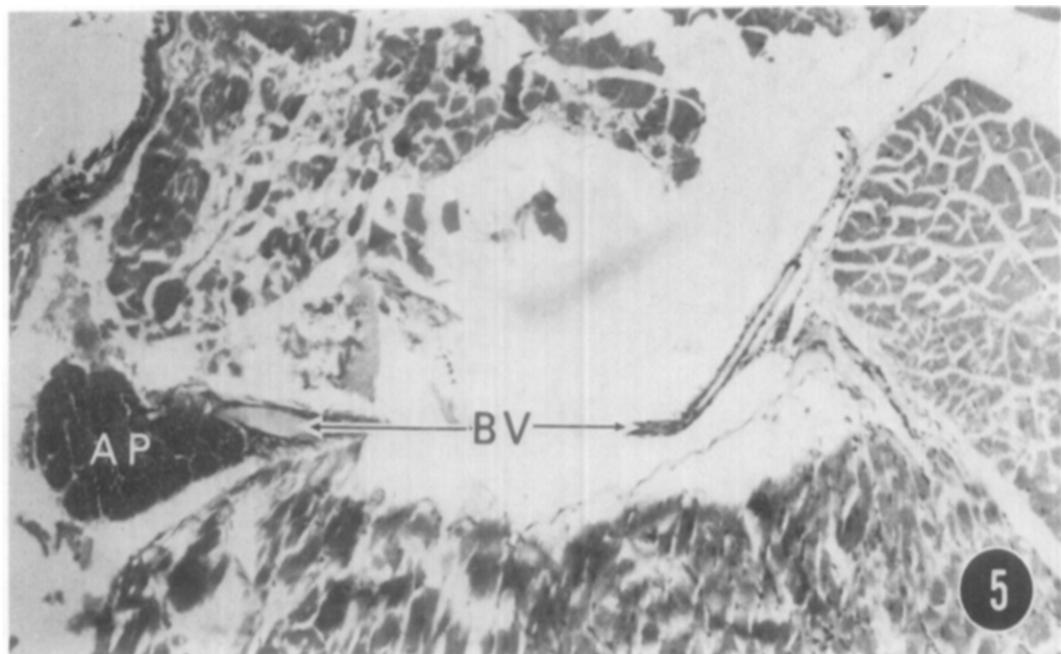


FIG. 5. *Agmasoma penaei* spores in muscle tissue adjacent to an intersegmental blood vessel. AP, *Agmasoma penaei*; BV, blood vessel. $\times 60$.

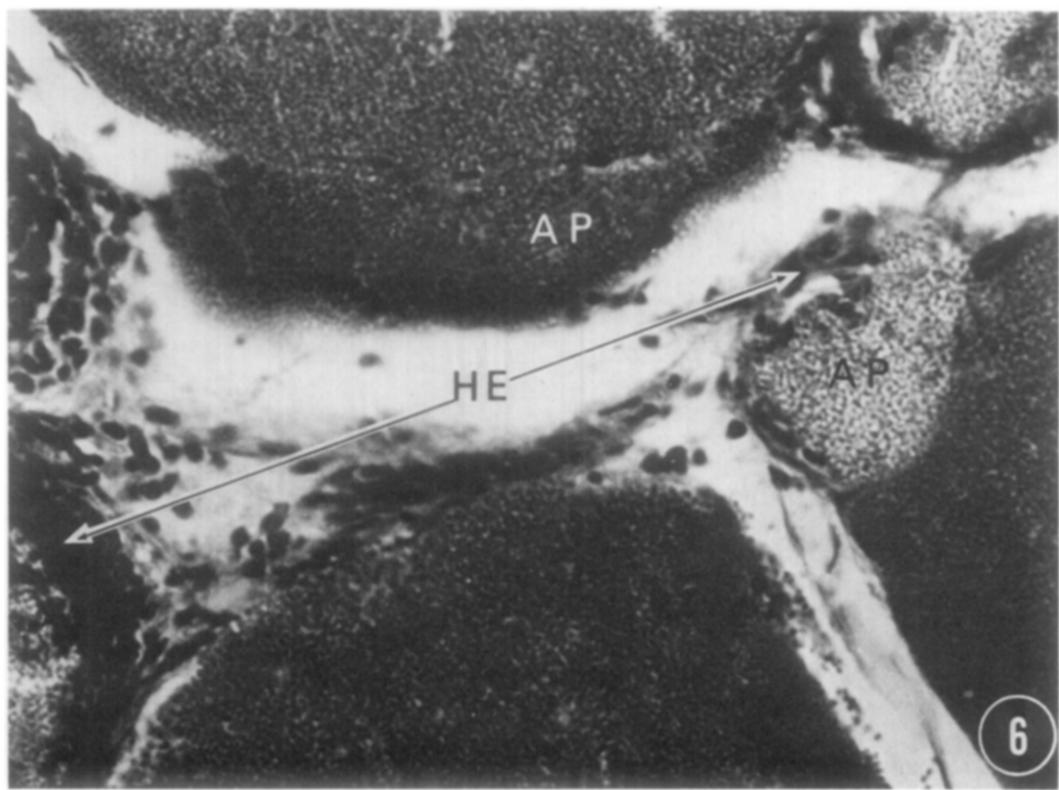


FIG. 6. Masses of *Agmasoma penaei* spores in abdomen. Note the capsule of hemocytes around the lighter-stained spore mass. AP, *Agmasoma penaei* spores; HE, hemocytes. $\times 450$.

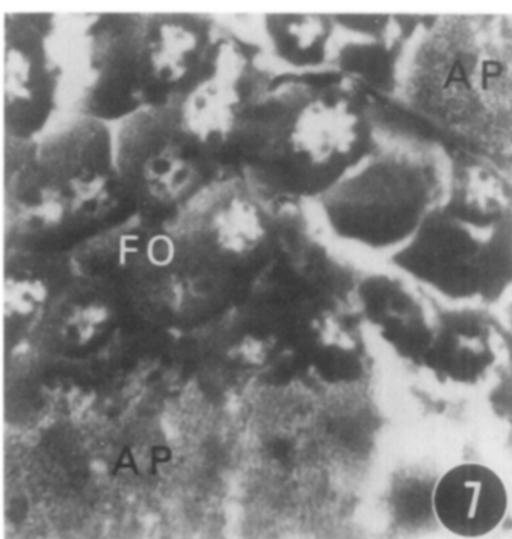


FIG. 7. Ovarian tissue infected with *Agmasoma penaei*. The few remaining immature follicles are surrounded by areas where follicles have been replaced by spores. AP, *Agmasoma penaei* spores; FO, follicles, $\times 485$.

thorax heavy infections of *A. penaei* occurred in digestive gland tissue. Much of the hemocoel normally occupied by digestive gland tissue became completely filled with spores in heavily infected specimens. Muscles adjacent to the digestive gland were lightly infected.

Ovaries. *A. penaei* infected entire ovaries, eventually completely destroying oocytes, connective tissue, and epithelium. Only small, residual portions of ovaries were observed in infected specimens (Fig. 7).

Pleistophora sp.: Gross Pathologic Manifestations

Pleistophora sp.-infected pink shrimp displayed gross symptoms identical to the "cotton" or "milk" conditions associated with *T. duorara* infection.

Pleistophora sp.: Tissues Infected.

Nearly all striated muscles in *Pleistophora*-infected shrimp contained spores. In the muscles that were infected, all secondary muscle bundles contained spores (Fig. 8). Heaviest infection occurred near the periphery of muscle bundles, with only

light infection at the interior of muscle fascicles. Muscle interstices were nearly devoid of spores (Fig. 9). Whole muscles or muscle fascicles were never completely destroyed. The extent of infected muscles and muscle fascicles varied, but usually less than one-half of the muscle tissue was replaced by spores.

The superficial dorsal abdominal muscles of *Pleistophora* sp.-infected shrimp were atrophied. The size of muscle bundles was greatly reduced and muscles appeared to be infiltrated with connective tissue.

Other infection sites. Only light infections were observed in the digestive gland, and spores were almost completely absent from the hemocoel. Small masses of *Pleistophora* sp. spores were observed within heart muscle and gill epithelium. In both of these areas large ($12 \times 5.5 \mu\text{m}$), ovoid, granular hemocytes were visible in close proximity to spores.

DISCUSSION

Through stress or injury penaeid shrimp may undergo muscle necrosis that often mimics the gross pathologic manifestations of microsporidiosis (Rigdon and Baxter, 1970). Ultrastructural studies (Kelly, unpubl.) confirm that no infectious agent is involved in this condition. *A. penaei* infections in pink shrimp, sometimes restricted to small foci in the abdomen, can easily be confused with muscle necrosis induced by injury.

The specific patterns of tissue infection exhibited by *A. penaei*, *T. duorara*, and *Pleistophora* sp. are additional characteristics for use in identifying these pathogens in pink shrimp.

Muscle atrophy observed in microsporidian-infected pink shrimp was mainly associated with *Pleistophora* sp. infection. Noticeable differences in behavior were seldom apparent in infected shrimp. Evidently a significant amount of muscle function was retained despite heavy microsporidian infection.

The pattern of muscle infection was related to the extent of damage done to mus-

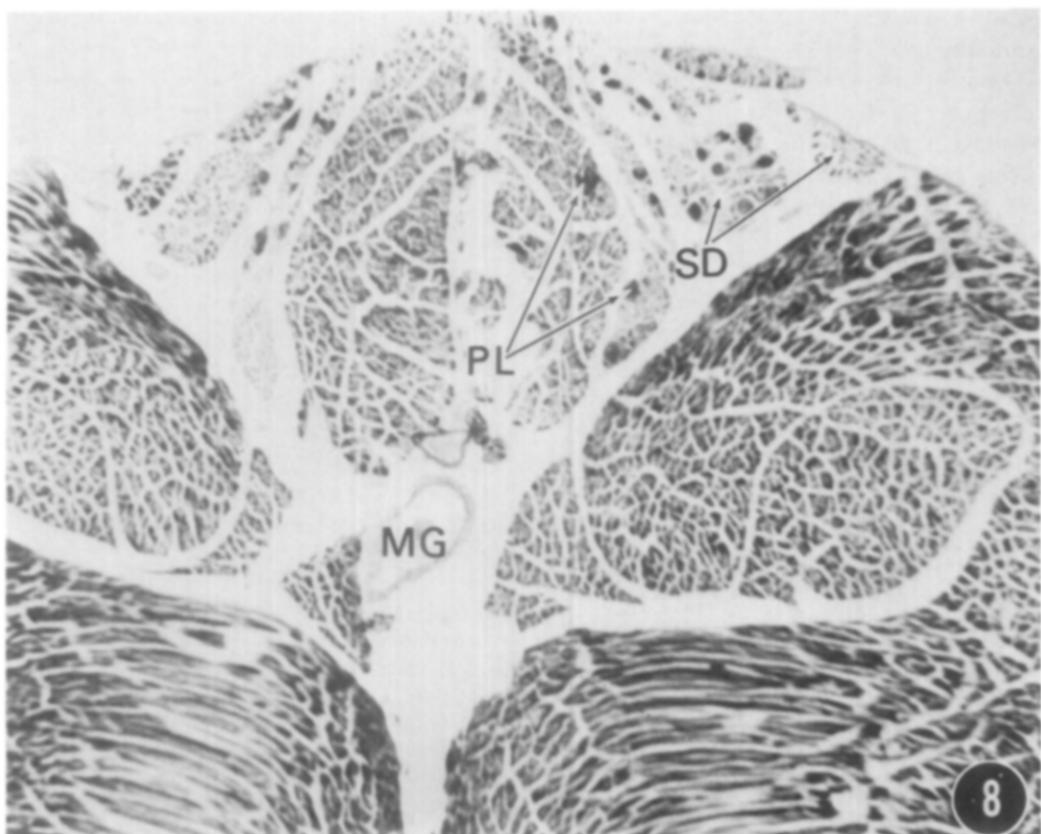


FIG. 8. Cross section of abdomen infected with *Pleistophora* sp. Note atrophy of superficial dorsal abdominal muscles. MG, midgut; PL, *Pleistophora* sp. spores; SD, superficial dorsal abdominal muscle. $\times 35$.

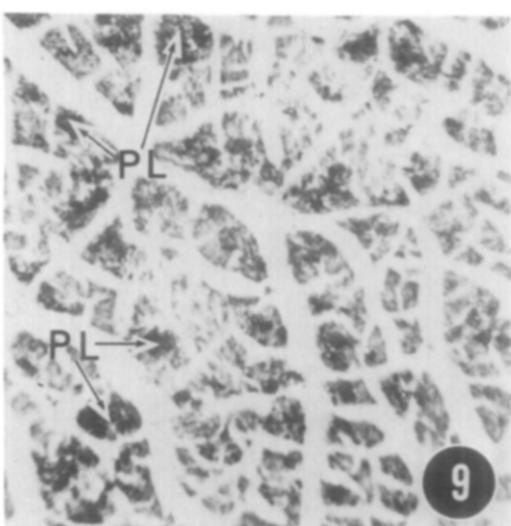


FIG. 9. Abdominal muscle infected with *Pleistophora* sp. Note the location of spores within partially infected muscles. PL, *Pleistophora* sp. spores. $\times 300$.

cle tissue by microsporidian infection. *T. duorara* infections were widely diffused throughout muscle interstices, but little, if any, lysis of muscle fibers was observed. *Pleistophora* sp. infections were widespread, but only infected a small portion of muscle fibers within individual muscle fascicles. Infections of *A. penaei*, which completely destroyed individual muscles, were confined to specific areas.

The lives of *A. penaei*-infected shrimp may have been prolonged by the fact that muscles most important to survival were among the last to become infected, if they are infected at all. *A. penaei* infections appeared to begin in the dorsal and lateral regions of the abdomen. The anterior oblique muscles, responsible for the strong abdominal flexion used in escape by penaeid

shrimp (Young, 1959), were never observed to be infected.

Descriptions of the pathology of *A. penaei* infections in white shrimp provided by Overstreet (1973) indicated differences in tissue specificities between infection in white and pink shrimp. Overstreet reported infection of the smooth muscle of blood vessels and proposed this tissue as the source of striated muscle infection. Although spores were often associated with striated muscle near blood vessels of pink shrimp, in this study blood vessels themselves were not observed to be infected.

In white shrimp from Georgia, Overstreet (1973) reported that *A. penaei* spores were present "around the fibers of the dorsal abdominal muscles." This condition is opposite to *A. penaei* infection of pink shrimp where, as shown in this study, spores were present within muscle bundles and replaced muscle fibers. Infection of ovaries of pink shrimp is in agreement with specificity of *A. penaei* for ovarian tissue of white shrimp (Sprague, 1950; Overstreet, 1973).

Detailed descriptions of *Pleistophora* sp. infections in brown and white shrimp made by Contransitch (1970) are very similar to what was observed in this study of pink shrimp. In both cases replacement of portions of individual muscles by spores occurred throughout the abdomen and thorax.

Host responses to microsporidian infection have been demonstrated previously in amphipods (Pixel-Goodrich, 1929), crayfish (Vey and Vago, 1973), and crangonid shrimp (Breed and Olsen, 1977). Unlike previous reports in which hemocyte responses were observed mainly in heavily infected specimens, encapsulation of *A. penaei* spores was most commonly noted in lightly infected pink shrimp. The latter case may indicate a more successful resistance to invasion by *A. penaei*. Staining differences of encapsulated *A. penaei* spores indicated possible physiological changes in the pathogen. *T. duorara*- and *Pleistophora* sp.-infected shrimp, which never exhibited

a host response, were always observed to be heavily infected.

The location of *T. duorara* spores and pansporoblasts at the periphery of muscle bundles and in the hemocoel adjacent to the digestive gland raises the possibility that this microsporidian may be extracellular during its final stages. Pérez (1927) previously identified *T. paguri*, a parasite of the hermit crab, *Eupagurus bernhardus*, as a "Microsporidie coelomique." Sprague et al. (1968) also proposed extracellular stages for *Ameson michaelis* in the blue crab, *Callinectes sapidus*.

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REFERENCES

- BREED, G. M., AND OLSON, R. E. 1977. Biology of the microsporidian parasite *Pleistophora crangoni* n. sp. in three species of crangonid sand shrimp. *J. Invertebr. Pathol.*, 30, 387-405.
- CONTRANSITCH, M. J. 1970. "Description, Pathology and Incidence of *Plistophora penaei* n. sp. (Microsporidia, Nosematidae), a Parasite of Commercial Shrimp." M.S. Thesis, Northwestern State University, Natchitoches, La.
- HAZARD, E. I., AND OLDACRE, S. W. 1975. Revision of Microsporidia (Protozoa) close to *Thelohania* with descriptions of one new family, eight new genera, and thirteen new species. *U.S. Dept. Agr. Tech. Bull.*, 1530, 1-104.
- IVERSEN, E. S., AND MANNING, 1959. A new microsporidian parasite from the pink shrimp (*Penaeus duorarum*). *Trans. Amer. Fish. Soc.*, 88, 130-132.
- KELLY, J. F. 1975. "A Description of the Histological Structure of Normal and Microsporidian-Infected pink shrimp, *Penaeus duorarum* Burkenroad." Ph.D. Thesis, University of Miami, Miami, Fla.
- MIGLARESE, J. V., AND SHEALY, M. H. 1974. Incidence of microsporidian and trypanorhynch cestodes in white shrimp, *Penaeus setiferus* Linnaeus in South Carolina estuaries. *S.C. Acad. Sci. Bull.*, 36, 93.
- OVERSTREET, R. M. 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture*, 2, 105-140.
- PÉREZ, C. 1927. Notes sur les epicarides et les rhizocephales des côtes de France. I. Sur l'"*Eupagurus bernhardus*" et sur quelquesuns de ses parasites. *Bull. Soc. Zool. Fr.*, 52, 99-104.
- PIXELL-GOODRICH, H. 1929. Reactions of *Gammarus* to injury and disease, with notes on some microspor-

- idial and fungoid diseases. *Quart. J. Microsc. Sci.*, **72**, 325–353.
- RIGDON, R. H., AND BAXTER, K. N. 1970. Spontaneous necroses in muscles of brown shrimp, *Penaeus aztecus* Ives. *Trans. Amer. Fish. Soc.*, **99**, 585–587.
- SINDELMANN, C. J. 1970. "Principal Diseases of Marine Fish and Shellfish." Academic Press, New York.
- SOGANDARES-BERNAL, F. 1962. Presumable microsporidiosis in the dwarf crayfishes *Cambarellus puer* Hubbs and *C. shufeldti* (Faxon) in Louisiana. *J. Parasitol.*, **48**, 493.
- SPRAGUE, V. 1950. Notes on three microsporidian parasites of Decapod Crustacea of Louisiana coastal waters. *Occas. Pap. Mar. Lab. La. State Univ.*, **5**, 1–8.
- SPRAGUE, V., VERNICK, S. H., AND LLOYD, B. J., JR. 1968. The fine structure of *Nosema* sp. Sprague, 1965 (Microsporida, Nosematidae) with particular reference to stages in sporogony. *J. Invertebr. Pathol.*, **12**, 105–117.
- SUMARI, O. 1970. Crayfish parasite in Finland. *FAO Fish. Bull.*, **2**, 12.
- VEY, A., AND VAGO, C. 1973. Protozoan and fungal diseases of *Austropotamius pallipes* Lereboullet in France. In "Freshwater Crayfish" (S. Abrahamson, ed.), pp. 165–179. Lund, Studnetlitt.
- VIOSCA, P. 1945. A critical analysis of practices in the management of warm-water fish with a view to greater food production. *Trans. Amer. Fish. Soc.*, **73**, 274–283.
- YOUNG, J. H. 1959. Morphology of the white shrimp *Penaeus setiferus* (Linnaeus 1758). *U.S. Fish Wildl. Serv. Fish. Bull.*, **59**, 1–168.