AN ABSTRACT OF THE THESIS OF

Gail Miner Breed for the degree of Master of Science

in Zoology presented on September 16, 1976

Title: BIOLOGY OF THE MICROSPORIDAN PARASITE, PLEISTOPHORA SP., IN THREE SPECIES OF CRANGONID SAND SHRIMP

Abstract approved: Redacted for Privacy

Robert E. Olson

The microsporidan Pleistophora sp. is a common parasite of Crangon franciscorum, C. nigricauda, and C. stylirostris in the vicinity of Yaquina Bay, Oregon. Characteristics of the parasite are described. Skeletal muscle was the only host tissue infected.

The seasonal prevalence and intensity of the parasite in crangonids are described, based on examination of 1,556 C. franciscorum, 3,877 C. nigricauda, and 1,674 C. stylirostris collected at monthly intervals from June, 1975, through June, 1976. Prevalence in C. franciscorum and C. stylirostris increased through the fall and reached winter peaks of 30.3% and 41.0% respectively, then decreased in the spring. Prevalence in C. nigricauda remained below 8% through the year. Intensity increased with size of the shrimp in the three species.

Infection experiments and field observations indicate that only very young shrimp are susceptible to infection during a relatively

short period during the summer months. Following initial exposure, the infection spread within the host, indicating repeated schizogonic cycles.

Parasitic castration was indicated by the absence of gravid infected female shrimp and was confirmed by histological examination. Ovaries of infected shrimp did not develop beyond a very early stage. A shift in sex ratio toward females in infected shrimp also indicates that the parasite may influence sex determination.

Shrimp showed little cellular response to infection. Only rarely in heavily infected shrimp was encapsulation of the parasite cysts observed, and necrotic tissue was occasionally observed.

Infected shrimp succumbed before uninfected shrimp under low oxygen stress. The collection of unusually large infected shrimp indicates that these shrimp either experienced accelerated growth or lived longer than uninfected shrimp.

Biology of the Microsporidan Parasite, *Pleistophora* sp., in Three Species of Crangonid Sand Shrimp

by

Gail Miner Breed

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed September 1976

Commencement June 1977

APPROVED:

Redacted for Privacy

Assistant Professor of Zoology
in charge of major

Redacted for Privacy

Acting Chairman of Department of Zoology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented September 16, 1976

Typed by Mary Jo Stratton for Gail Miner Breed

ACKNOWLEDGEMENTS

To Michael, Marilyn, Cathy, John, and Range, who never quite succeeded in trapping the entire Pacific Ocean in their hip waders, for the blisters, the aching muscles, tired and soaked bodies, midnight oil, a dirty joke or two, and the undying determination to help;

to William Breese and Bob Malouf for sharing countless gallons of Pseudoisochrysis soup;

to my major professor, Bob Olson, who never realized that without his gentle prodding and patience, guidance and understanding, time, energy, and editing, this study could never, would never have been completed;

and to myriad other students, staff members, family and friends who gave their unsolicited interest, time, and attention . . .

Thank you

TO GEORGE

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

BIOLOGY OF THE MICROSPORIDAN PARASITE, PLEISTOPHORA SP., IN THREE SPECIES OF CRANGONID SAND SHRIMP

INTRODUCTION

Crangonid shrimps are an important component of the decapod shrimp fauna that inhabits Oregon's coastal waters. They contribute substantially to nutrient cycling and are a predominant food item for many marine fishes (Haertel and Osterberg, 1966; Krygier and Horton, 1975). Three species of crangonids, Crangon franciscorum (Stimpson), C. nigricauda (Stimpson), and C. stylirostris (Holmes), found in the vicinity of Yaquina Bay, Oregon, are parasitized by the microsporidan, Pleistophora sp. The biology of two of these, C. franciscorum and C. nigricauda, was studied by Krygier and Horton (1975).

Microsporidans are protozoan parasites of members of most invertebrate phyla and also occur in some vertebrates. They were first defined by Balbiani (1882) as being intracellular, having spores contained within a packet, and having spores with a polar capsule containing one or two coiled polar filaments.

This definition has been modified by various workers to incorporate the results of later studies (Kudo, 1924; Corliss and Levine, 1963; Honigberg, Balamuth, Bovee, Corliss, Gojdics, Hall,

Kudo, Levine, Loeblich, Weiser, and Wenrich, 1964; Sprague, 1969). The most recent taxonomic revision is that of Levine (1970), who, like Sprague (1969), raised the Microspora to subphylum rank, distinguishing it from the subphyla Myxospora Sprague, 1969, and Apicomplexa Levine, 1970. Presently the Microspora are characterized as having spores of unicellular origin; having a single sporoplasm and single valve (Honigberg et al., 1964; Levine, 1970). Members of the Class Microsporea Corliss and Levine, 1963, possess a tubular polar filament.

Genera of the Order Microsporida are differentiated by the number of spores that develop from a sporont following sporogony. The genus Pleistophora Gurley, 1893, is defined as having 16 or more sporoblasts per sporont, each of which becomes a spore. Until recently, pleistophorans were included in the family Nosematidae Labbe, 1899, because they have oval or pyriform spores and a single polar filament. Street and Sprague (1974) revived the family Pleistophoridae Stempell, 1909 (genera having a variable, usually large number of spores contained within a pansporoblastic membrane), which now includes the genus Pleistophora.

Five species of Pleistophora have been described as parasites of decapod crustaceans: Pleistophora cargoi Sprague, 1966, in the skeletal and cardiac muscles of the crab Callinectes sapidus Rathbun, 1896 (Sprague, 1966); P. lintoni Street and Sprague, 1974, in the

muscles of the grass shrimp, Palaemonetes pugio Holthius (Street and Sprague, 1974); P. miyiarii Kudo, 1924, in the gut of the freshwater shrimp Atyephira sp. (Kudo, 1924); P. sogandaresi Sprague, 1966, in the muscles of the crayfish Cambarellus puer Hobbs (Sprague, 1966); and Pleistophora sp. (Baxter, Rigdon, and Hanna, 1970; Contransitch, 1970) in the muscle, pericardium, hepatopancreas, and stomach wall of the commercial shrimps, Penaeus aztecus Ives and P. setiferus (L.).

The bulk of the studies on the microsporidans of decapods are taxonomic in nature; investigations into the biology of these parasites are few. Weidner (1970) studied the development of Nosema nelsoni Sprague, 1950, in Callinectes sapidus and succeeded in experimentally infecting the crabs; Constransitch (1970) studied Pleistophora sp. in two species of penaeid shrimp.

Until recently, all attempts to experimentally transmit microsporidans to shrimp have failed. However, Iversen and Kelly (1976) have now successfully infected the shrimp, Penaeus duorarum Burkenroad with Thelohania sp. by allowing spores to pass through the digestive tract of a fish prior to feeding to shrimp.

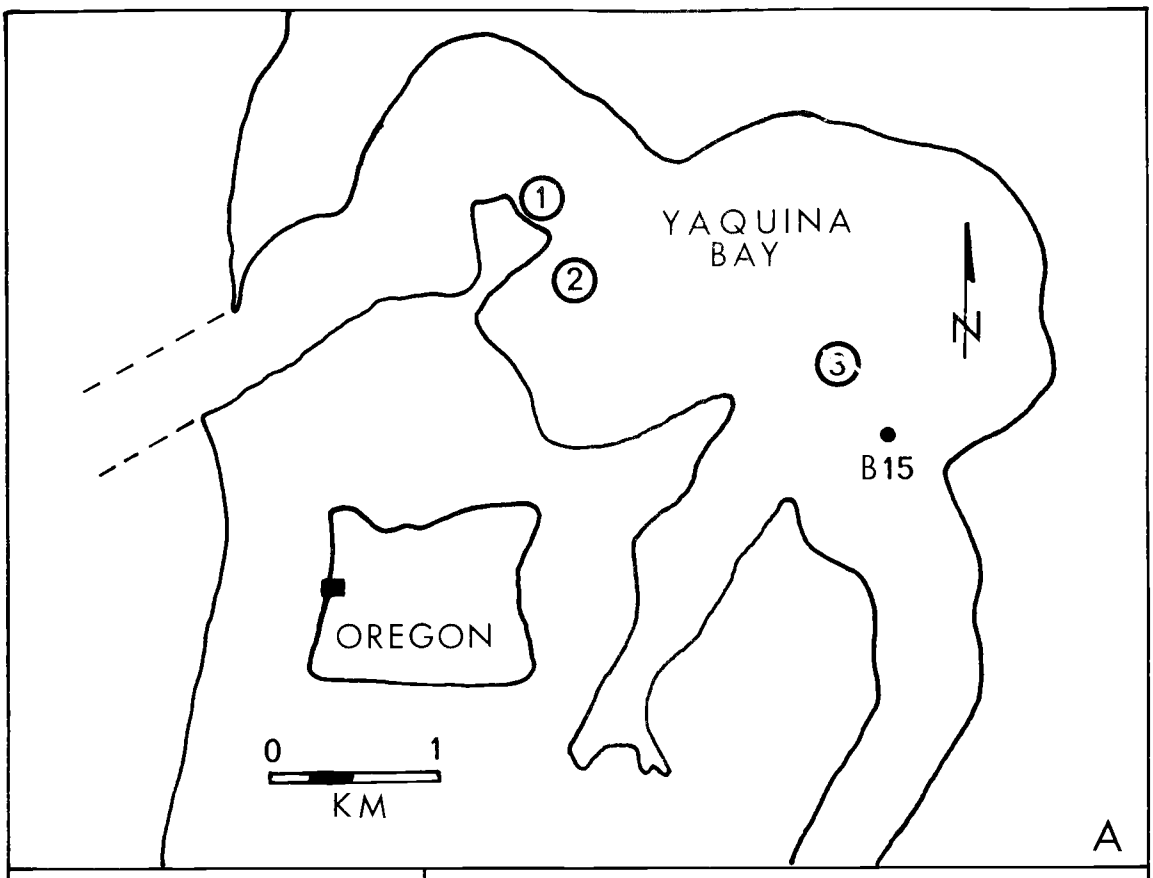
The purpose of this study was to describe the seasonal variations in prevalence and intensity of the parasite in Crangon spp. populations, the histopathological effects of the parasite, and the

effects on the shrimp in terms of fecundity, capacity to withstand stress and mortality. It was also hoped that the mode of parasite transmission could be determined.

STUDY AREAS AND METHODS

Crangonid shrimps were collected monthly from June, 1975, through June, 1976, from three areas in Yaquina Bay and one at Seal Rock, Oregon (Fig. 1). Stations 1 and 2 were located on tidal mud flats and station 3 was a subtidal area with a substrate that consisted of muddy sand and shells. Both Crangon franciscorum and C. nigricauda were collected at these stations. Station 4 at Seal Rock was an open coast sandy beach area with rocky outcroppings. Only C. stylirostris was collected at station 4.

Three different shrimp collection methods were employed, depending on the station. Samples from stations 1 and 2 were obtained by pulling a beach seine (stretch mesh 1.0 cm) in 1-2 ft of water. A 16 ft semi-balloon trawl was used to sample at station 3. The net body consisted of 3.8 cm stretch mesh (s.m.) with a cod end of 3.2 cm s.m. and a 1.3 cm s.m. liner. The trawl was pulled on the bottom for approximately 15 min by the R/V Paiute or by the dory Redi. Samples were usually collected at low tide when the channel depth was approximately 12 m. Samples from station 4 were collected with a rectangular dip net that was pushed along the bottom in the surf and in sandy-bottomed tide pools. Unfavorable tidal, weather, and sea conditions made uniform sampling difficult. Nonetheless, most



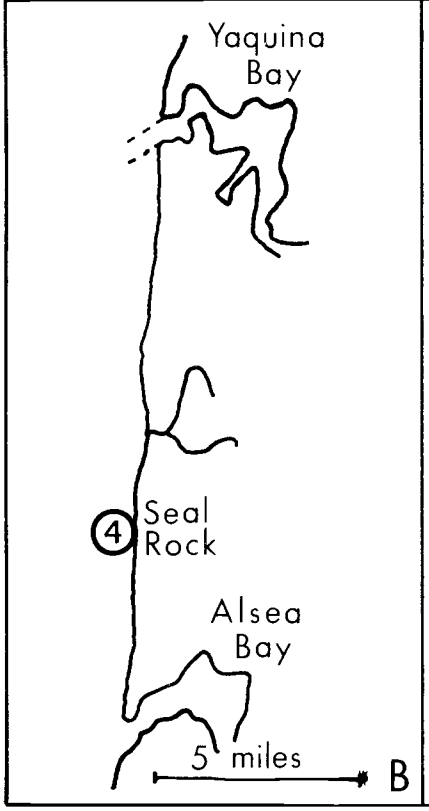


Figure 1.

Sampling stations. A. Yaquina Bay stations 1, 2, and 3. B. Location of Seal Rock station in relation to Yaquina Bay.

samples contained at least 100 shrimp and the period between monthly samples was always at least 20 days.

The shrimp were held in tanks of running seawater at the Oregon State University Marine Science Center until examined. Each shrimp was measured to the nearest mm from tip of rostrum to tip of telson (total length: TL) and sex was determined by examination of secondary sexual characteristics (Meredith, 1952). The presence or absence and degree of microsporidan infection was determined by examining each shrimp under a dissecting microscope. Infections were qualitatively categorized as light, medium, or heavy according to the following criteria (Fig. 2):

Light: abdominal musculature contained a few scattered streaks of infected tissue that were an opaque white in appearance;

Medium: approximately half of the abdominal musculature appeared white and opaque;

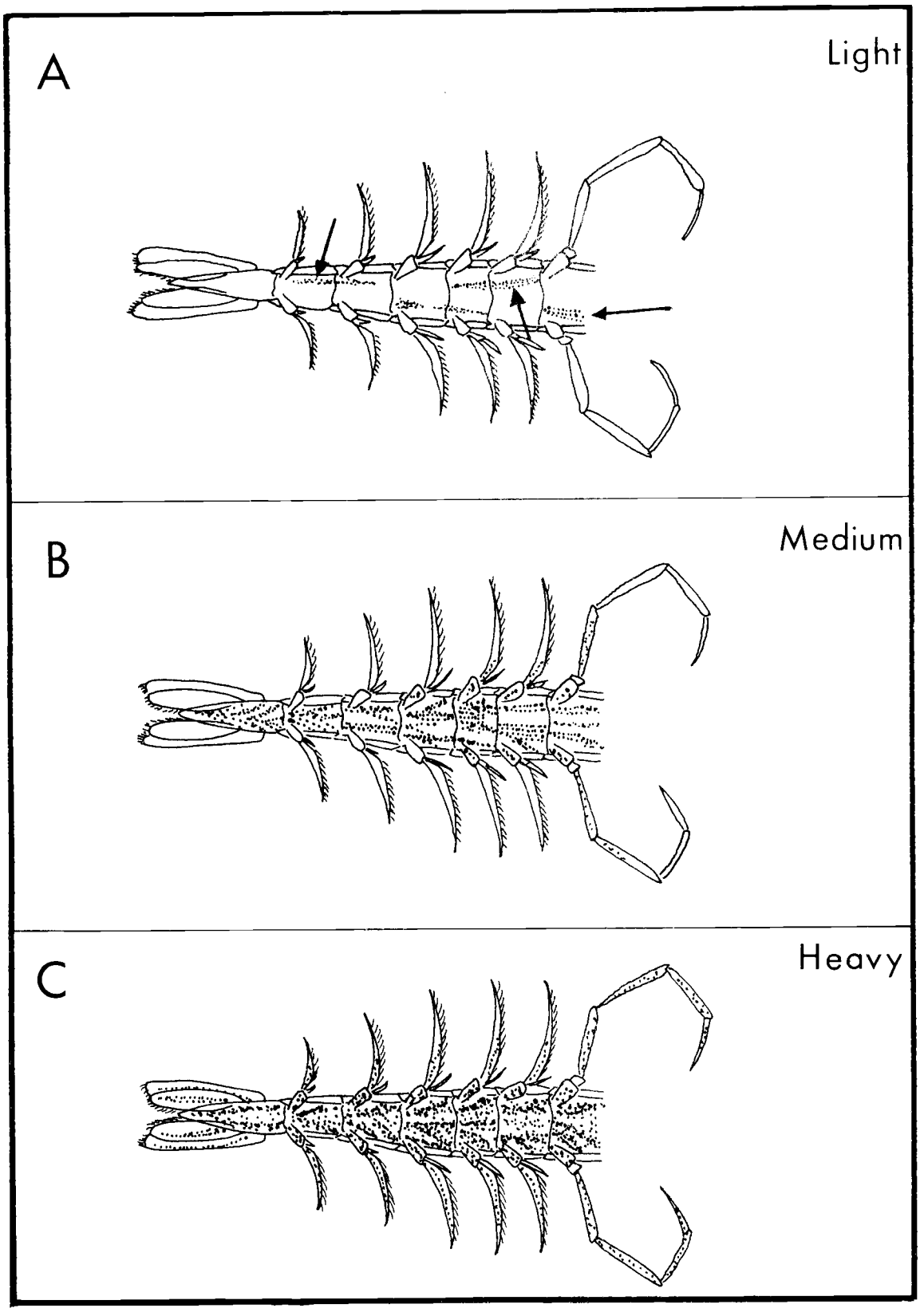
Heavy: nearly all of the muscle tissue visible under the microscope appeared white and opaque.

At least ten shrimp from each sample were fixed in Bouins solution and embedded in Paraplast® for sectioning at 7-10 μm. Sections were stained in hematoxylin and eosin, mounted in synthetic resin and examined under a compound microscope.

Fresh spores were obtained by crushing infected tissue under a coverslip and were studied with a phase contrast microscope. For

Figure 2. Drawings illustrating the intensity of infection indicated by the terms light, medium, and heavy infection.

A. Light infection. Note scattered patches (arrows).
B. Medium infection. About half of the visible muscle is opaque.
C. Heavy infection. Nearly all muscle is opaque.


A
Light
B
Medium
C
Heavy

photography, spore suspensions were sealed under a coverslip with clear fingernail polish and allowed to settle overnight before photographing with a Zeiss® C35 photomicrographic camera mounted on a Zeiss® Standard RA microscope with planachromat 40x/0.65 numerical aperture (n.a.) and 100x/1.25 n.a. objectives. Spores were measured by comparing photographs of spores with a photograph of a micrometer scale made at the same magnification. Cysts (pansporoblasts) were measured directly on the slide with a calibrated ocular micrometer.

To cause extrusion of the polar filament, spores were subjected to the following treatments or reagents as recommended by Kudo (1924): mechanical pressure, distilled water, iodine water, ammonia water, and methylene blue solution.

To study other spore characteristics, smears of infected muscle tissue were fixed in methanol, Bouins, or Schaudinns solutions. Schaudinn-fixed spores were treated by the Feulgen reaction. Following the other fixatives, alcoholic PAS (Humason, 1967), Giemsa, and Heidenhain's iron hematoxylin methods were applied.

Apparently uninfected shrimp to be used in infection experiments were held in tanks of circulating seawater and fed Oregon Moist Pellet (Hublou, 1963) for a minimum of 25 days before use. After this, shrimp were reexamined for infections and any infected shrimp were discarded.

Spores to be used in infection experiments were obtained by crushing infected skeletal muscle tissue in an homogenizer and suspending the released spores in filtered seawater. The spores were used immediately or stored at 6C or 20C for a maximum of 60 days before use. Potential vectors (Artemia salina) were placed in spore suspensions and allowed to feed upon the spores for at least 24 hr. Gut contents of the brine shrimp were always checked for the presence of spores before use. Brine shrimp carrying spores were washed several times in filtered seawater to remove uningested spores and were fed immediately to the experimental crangonid shrimp.

Following a modification of the methods of Iversen and Kelly (1976), six heavily infected Crangon stylirostris were fed to a sand sole (Psettichthys melanostictus) and the feces were collected for the next two days. Following confirmation of spore content, the feces were fed directly to experimental crangonids or were placed in suspension and fed to Artemia salina for 24 hr before feeding them to the crangonid shrimp.

Each experimental shrimp was placed in a 400 ml beaker of filtered seawater immersed in distilled water circulated through a controlled temperature bath. Following exposure to spores, the experimental shrimp were fed non spore-carrying Artemia salina daily. The temperature and duration of experiments varied as shown

in the results. To detect presence of infection, the shrimp were examined weekly under a dissecting microscope.

To determine the effect of infection on shrimp under low oxygen stress, nine heavily infected and nine uninfected Crangon stylirostris of similar size were individually placed in 400 ml, tightly covered beakers filled with 15C seawater saturated with oxygen. To monitor oxygen depletion, measurements of the dissolved oxygen in the water were taken every two hours with a YSI Model 54 Oxygen Meter. Monitoring was discontinued when the shrimp died or at the end of 46 hr. Smears of tissue from all uninfected shrimp were made following the experiment to determine the presence or absence of vegetative parasite stages not grossly visible. Infected shrimp were processed for routine histological study.

In an experiment to observe the progress of microsporidan infection, nine naturally infected Crangon stylirostris were isolated in 400 ml beakers filled with filtered seawater, held at seawater temperatures, and fed brine shrimp daily. Biweekly examination was made to characterize the spread of infection. The abdomen of each shrimp was visually divided into regions (Fig. 3) and the approximate percentage of apparent infection in each region was determined.

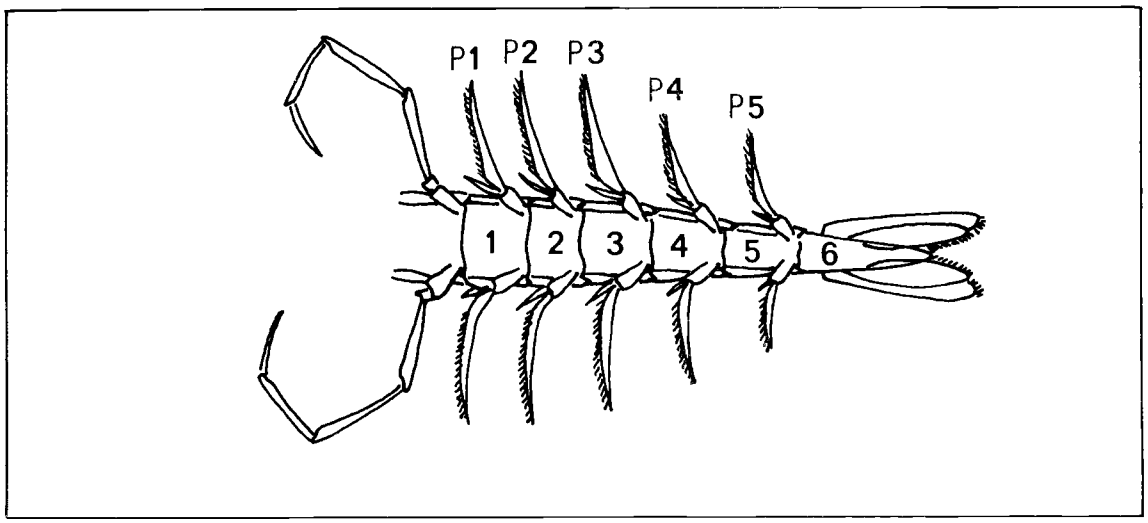


Figure 3. Abdominal regions of crangonid shrimp:

Area I = abdominal segments 1 and 2;
Area II = abdominal segments 3 and 4;
Area III = abdominal segments 5 and 6;
Area IV = pleopod pairs, numbered P1-P5.

At the termination of each experiment, all shrimp were fixed in Bouins solution and prepared for routine histological study.

To rear crangonid shrimp, newly hatched larvae (day 0) were placed in 400 ml or 1500 ml beakers or 2 gal aquaria and fed daily. Food items consisted of Artemia salina nauplii, Balanus sp. nauplii, unidentified zooplankters, and diatoms. Filtered seawater was changed every 1-2 days, allowing at least 40 ml per larva.

RESULTS

Description of Pleistophora sp.

Host species: Crangon franciscorum (Stimpson), C. nigricauda (Stimpson), C. nigromaculata (Lockington), C. stylirostris (Holmes).

Host tissue infected: Skeletal muscle.

Type locality: Yaquina Bay, Oregon; Seal Rock, Oregon.

Vegetative stages: Schizonts not positively identified.

Sporulation: Sporont gives rise to large number (50 to over 100) of sporoblasts enclosed within a pansporoblastic membrane. Each sporoblast becomes a spore.

Spore: Generally ellipsoidal with slight attenuation at the anterior end. Size: 2.4 x 1.4 μm. Membrane 0.1 μm thick and refractile. Posterior end contains clear vacuole. Anterior end shows PAS-positive polar cap. Polar filament also gives positive reaction to PAS and is coiled in the middle of the spore. Extruded polar filaments averaged 41.2 μm (25-62 μm) in length. Nucleome approximately centrally located.

Differentiating characters: Five species of Pleistophora have been described from decapods. Two of these, P. miyairii and P. sogandaresi, infect freshwater decapods, Atyephira sp. and Cambarellus puer respectively. The former is found in the gut of the shrimp and has large, ovoidal spores, 9 x 7 μm. The latter species, although found in the skeletal muscle of the crayfish host, differs from the one described here by having comma-shaped spores 6-9 x 4 μm in size. Pleistophora cargoi, found in the cardiac and skeletal muscle of the marine crab, Callinectes sapidus, has larger spores (5.1 x 3.3 μm) than the crangonid pleistophoran. Pleistophora lintoni, a parasite of the marine shrimp Palaemonetes pugio, infects the skeletal muscle and has ellipsoidal spores of 3.0 x 1.7 μm. Pleistophora sp. (Baxter, Rigdon, and Hanna, 1970; Constransitch, 1970) infects the skeletal and cardiac muscle, gills, stomach and hepatopancreas of other marine shrimp, Penaeus aztecus and P. setiferus. The spores are slightly larger (2.6 x 1.9 μm) than those of the present species and they are ovoid. The number of spores that develops from a sporont in the present species is similar to that from P. lintoni, P. cargoi, and Pleistophora sp., but is much greater than that from a sporont of P. sogandaresi (19-21).

The principal differentiating characters of the present Pleistophora species are its spore size and host. The geographic distribution of the host is separate from that of the hosts of previously

described species. The spores are the smallest reported for pleistophorans in decapods. The genus Crangon has previously been reported to host a microsporidan, but the parasite was in the genus Thelohania (Henneguy and Thelohan, 1893). The pleistophoran described here differs from P. miyiarii and Pleistophora sp. in site of infection and from P. miyairii, P. sogandaresi, and Pleistophora sp. in spore shape. It most closely resembles P. lintoni in infection site and spore shape, but is distinguished from it, as from the others, by size, host, and geographical location. On this basis, the species studied here is considered to be new.

Description: When infected shrimp were viewed with the unaided eye, or with a dissecting microscope, the infection appeared as white, opaque streaks that followed the long axis of the muscle fibers in the cephalothorax, abdomen, and appendages. When sections of infected tissue were observed with a compound microscope under low magnification, the infection appeared as rounded masses in cross section and elongated masses in longitudinal section. With high magnification, it became apparent that the masses were composed of multinucleate stages and both immature and mature spores (Fig. 4).

Detailed study showed that the multinucleate stages were located at the extreme edges of the infected tissue (Fig. 5). These ranged in size from very small (2.2 μm) binucleate bodies to larger (12.4 μm) multinucleate bodies between the muscle fibers. Only two intracellular stages of the parasite were observed (Fig. 6).

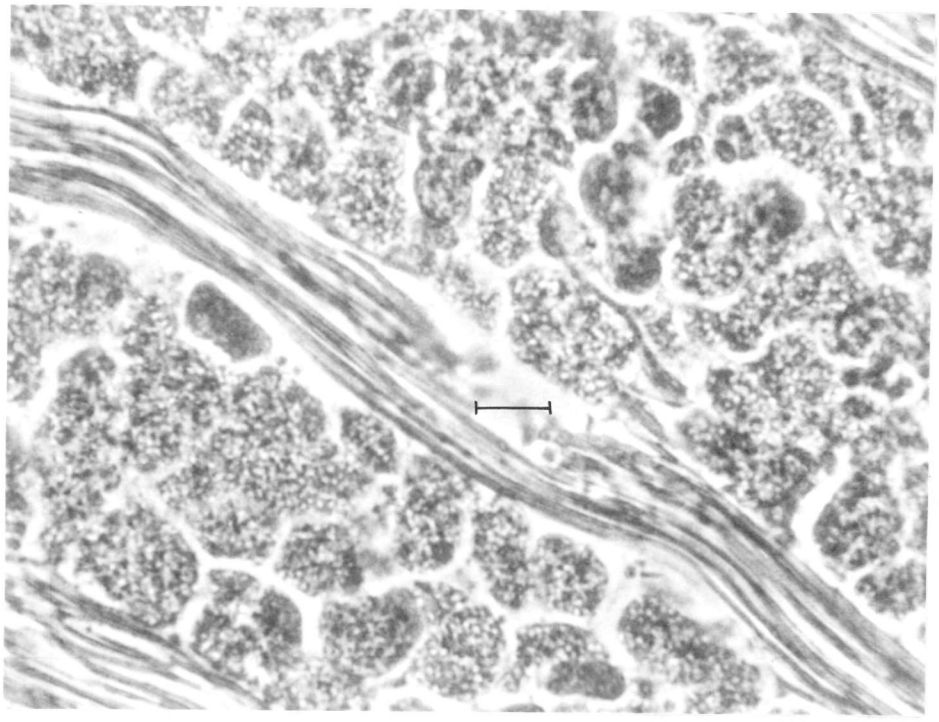


Figure 4. Longitudinal section of muscle from Crangon franciscorum heavily infected with Pleistophora sp. Scale = 10 μm.

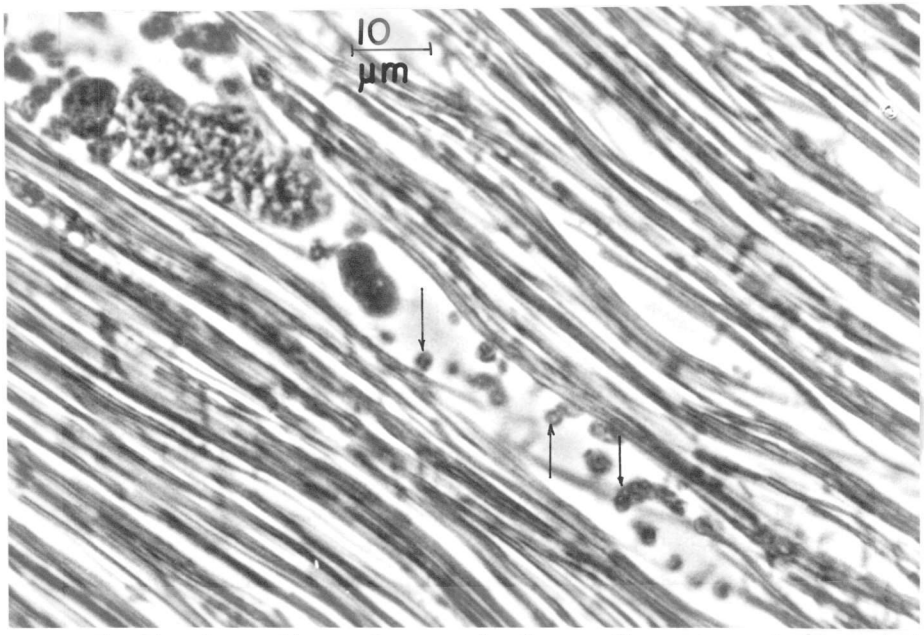


Figure 5. Longitudinal section of muscle from Crangon stylirostris, showing multinucleate parasite stages at edges of area of infection (arrows). Hematoxylin and eosin.

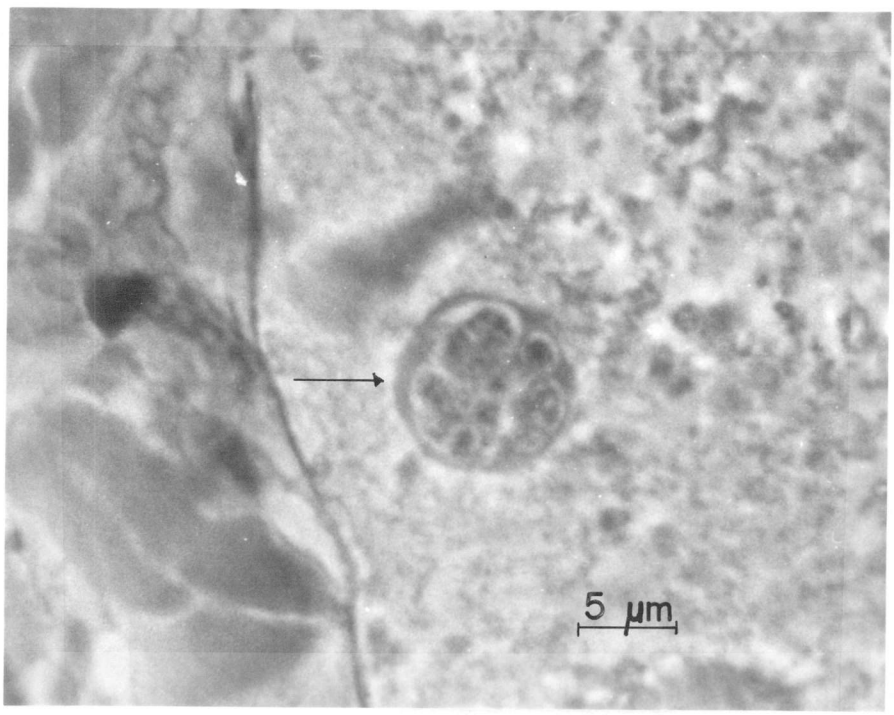


Figure 6. Intracellular stage of Pleistophora sp. in C. stylirostris (arrow). Hematoxylin and eosin.

Both sporoblasts and mature spores were observed within pansporoblastic membranes (Fig. 7). The cysts (pansporoblasts) were spherical, as small as 8.6 μm, or elongated to 32.9 μm (ave: 17.0 x 13.6 μm). The number of spores per cyst was always too large to permit accurate counting.

Fresh spores were uniformly ellipsoidal 2.1-3.2 x 1.2-2.0 μm (ave: 2.4 x 1.4 μm, 300 spores), with a slight attenuation at the anterior end (Fig. 8). Spores notably larger than the average were occasionally observed. Unstained spores had a conspicuous posterior vacuole and a spore membrane, about 0.1 μm thick, that was refractile when viewed with phase contrast illumination.

Polar filament extrusion was difficult to achieve. Nonetheless, 22 extruded filaments were measured and had a range of 25-60 μm in length (ave: 41.2 μm) (Fig. 9). The wide range may indicate that extrusion was not always complete.

Smears of spores stained with Giemsa showed a large nucleome and coiled polar filament. The spore membrane did not stain, but its presence was indicated by the clear area between the spore contents and the pale background.

Spores subjected to the Feulgen reaction showed a large, centrally located but indistinct nucleome (Fig. 10). The anterior and posterior areas of the spores remained clear. Presumably these areas contain the polaroplast and posterior vacuoles respectively.

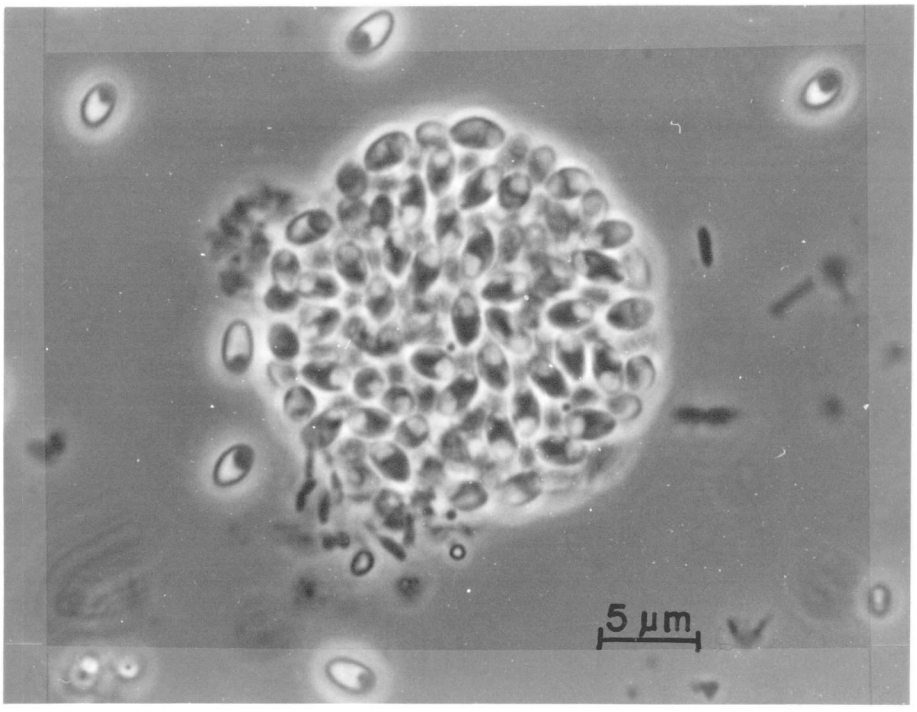


Figure 7. Cyst (pansporoblast) of Pleistophora sp. Fresh.

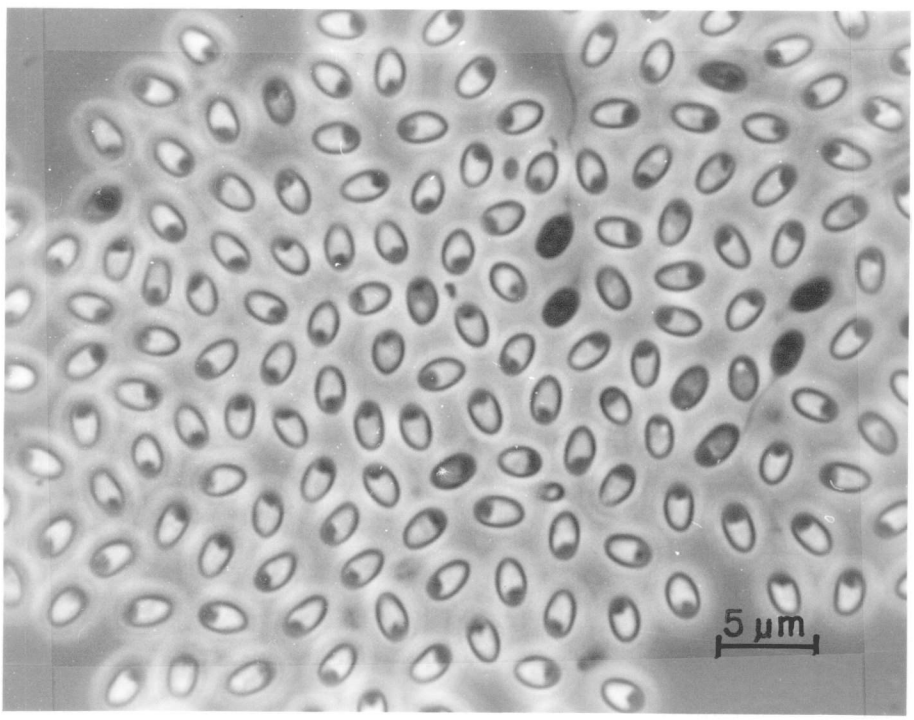


Figure 8. Spores of Pleistophora sp. Dark-colored spores have extruded polar filaments. Fresh.

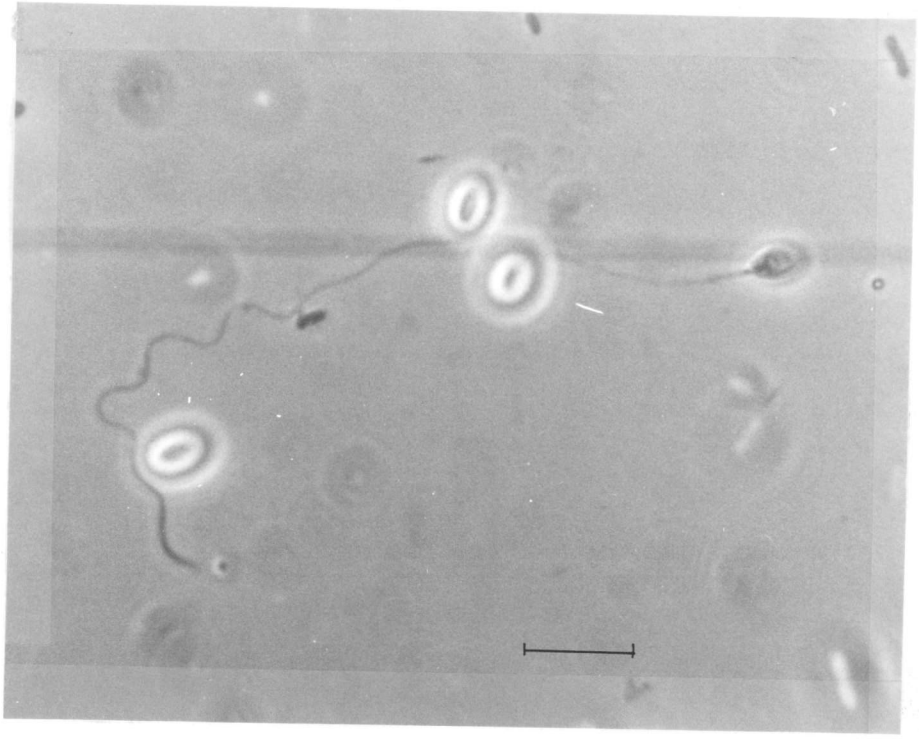
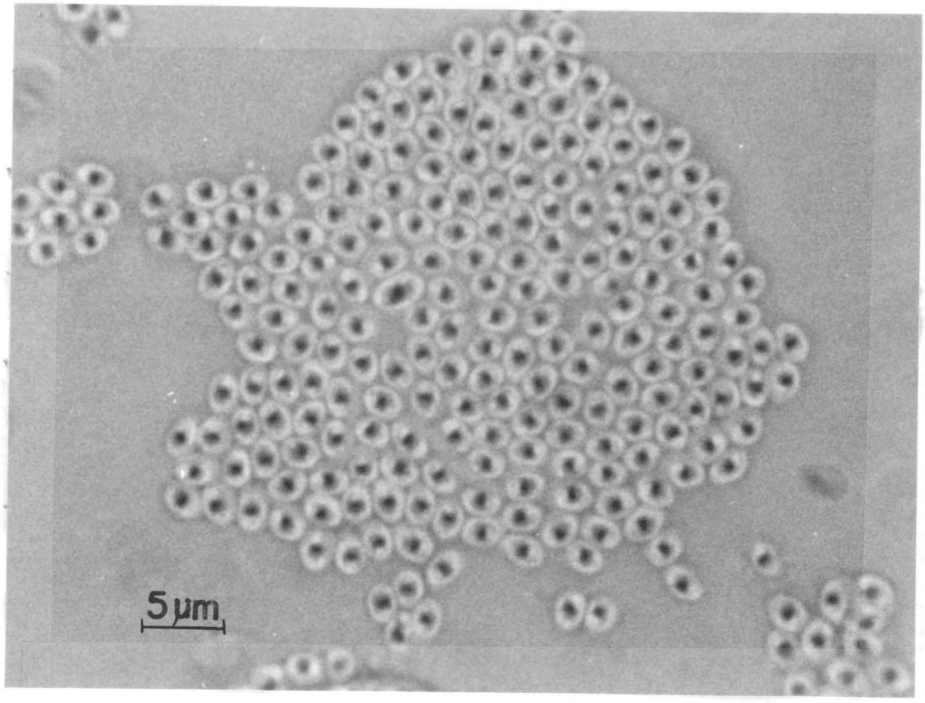
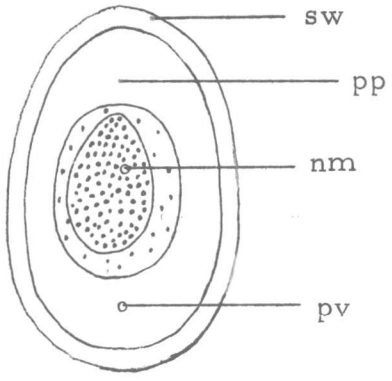


Figure 9. Polar filament extruded from Pleistophora sp. spore from Crangon nigricauda. Fresh. Scale = 5 μm.



A



B

Figure 10. Spores of Pleistophora sp. showing the Feulgen reaction. A. Photomicrograph of spore smear, showing dark reaction at center of spore. Note larger spore near center of group of spores. B. Drawing of Feulgen-treated spore. nm = nucleome; pp = polaroplast; pv = posterior vacuole; sw = spore wall.

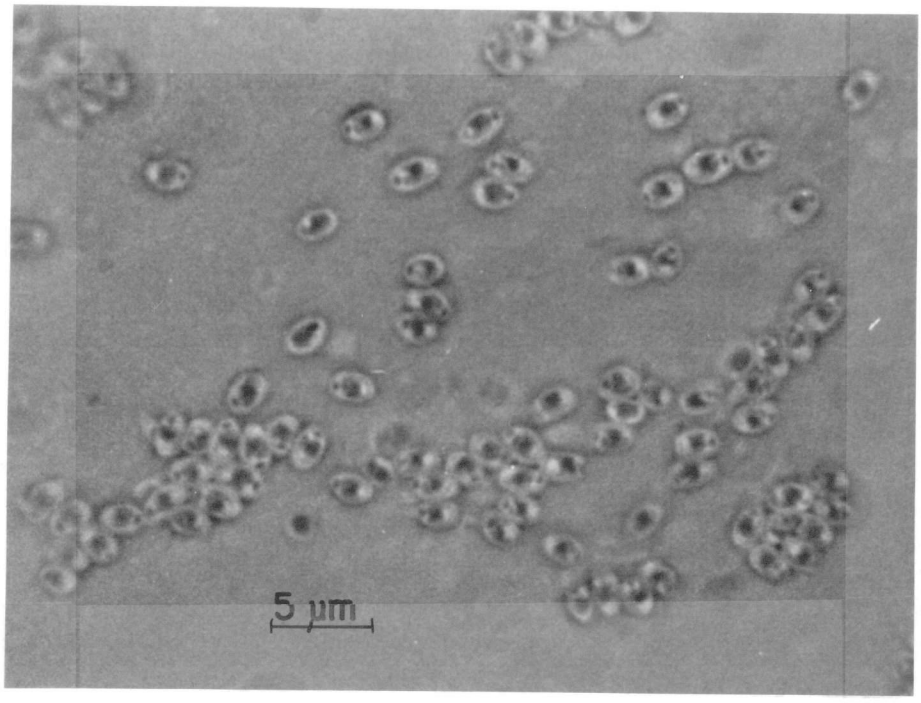
After the PAS treatment, the characteristic intense positive reaction appeared at the polar cap region. The polar filament gave a slightly positive reaction. It extended posteriorly from the polar cap then laterally and coiled to fill the remainder of the anterior two-thirds of the spore (Fig. 11). The number of coils of the filament was not visible. All other spore contents were PAS-negative.

Relative Abundance and Spawning Seasons of Crangon spp.

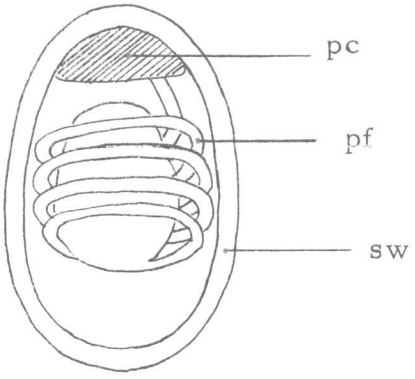
Crangon franciscorum

A total of 1,556 Crangon franciscorum was collected during the 13-month study. The relative proportions of each size class (4 mm increments) of C. franciscorum collected each month are shown in Figure 12. The shrimp were abundant in the sampling areas during the winter months, but not in the summer, making collection of large quantities of shrimp difficult in the latter period. The size of C. franciscorum collected varied throughout the year, the smallest individuals appearing in August (17 mm TL) and the largest in March (78 mm TL).

The spawning season for C. franciscorum extended from February, when 21.2% of 89 mature females were ovigerous, through July, when all eight mature females collected carried eggs. Recruitment of juveniles to the population occurred twice during the year, in



A



B

Figure 11. Spores of Pleistophora sp. showing the PAS reaction. A. Photomicrograph of spore smear, showing positive reaction in polar cap region and in area of polar filament. B. Drawing of PAS-treated spores. pc = polar cap; pf = polar filament; sw = spore wall.

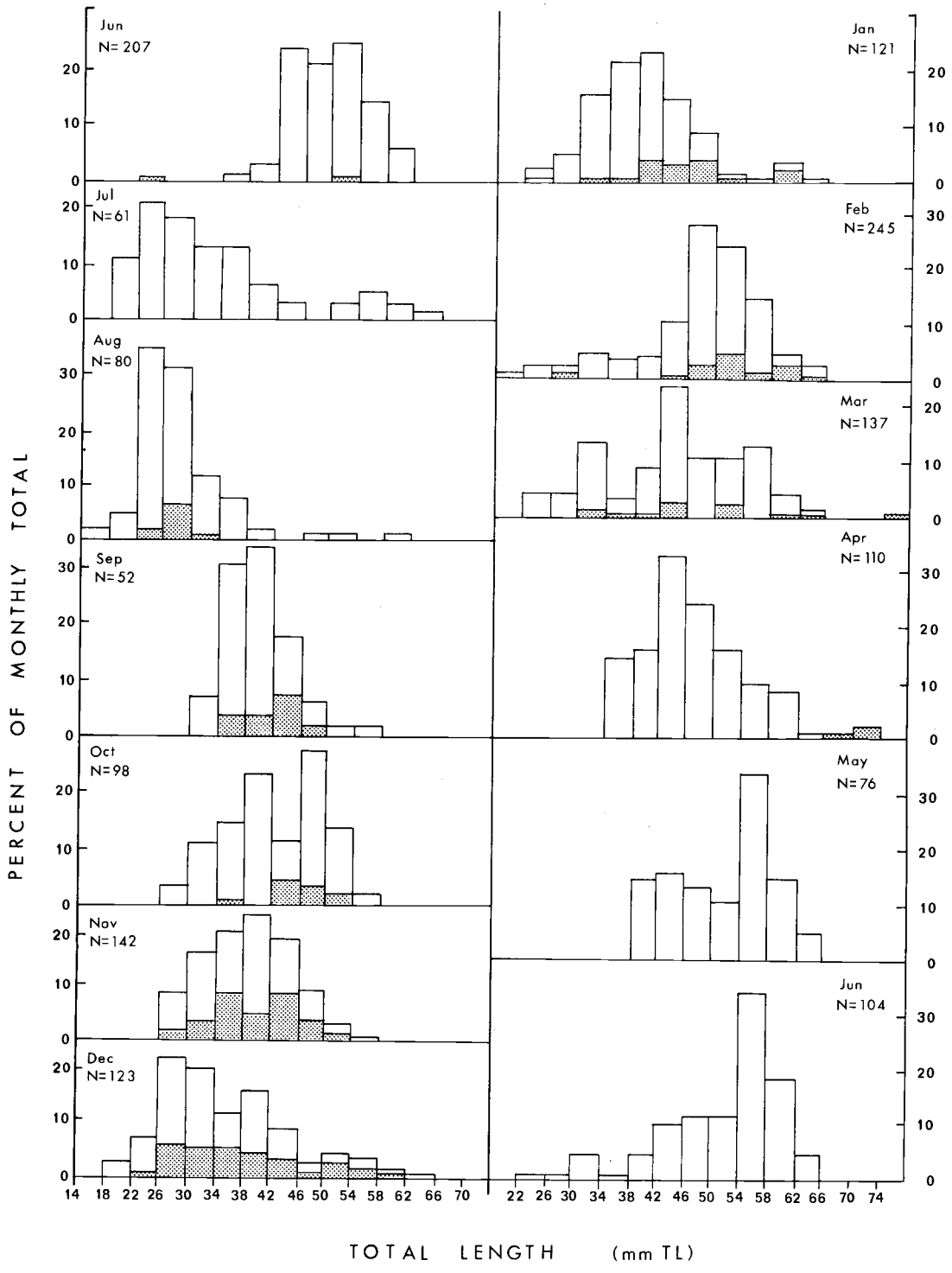


Figure 12. Length-frequency histograms of Crangon franciscorum in Yaquina Bay, Oregon, 1975-76. Clear bars = total animals; shaded bars = infected animals.

July through August and in December through February (Fig. 12), indicating that two relatively distinct periods of egg hatching occurred within the spawning season.

Crangon nigricauda

Crangon nigricauda outnumbered C. franciscorum approximately 2.5:1 in the samples collected from the same sampling areas. A total of 3,877 of C. nigricauda was captured during the study. Like C. franciscorum, C. nigricauda was abundant in the winter and less common in the late summer. A length-frequency diagram for each month's collection of C. nigricauda is shown in Figure 13. The smallest shrimp (12 mm TL) were collected in September, January, and February; the largest (61 mm TL) in May.

The spawning season began in February, when 37.5% of the mature females (N=32) were gravid, and continued into October. In September, 91.4% of 58 mature females carried eggs and 58.1% of 62 females were ovigerous in October. No gravid females were collected from November through January. Recruitment of juveniles occurred in July through September and in January through February (Fig. 13). This indicates that C. nigricauda also exhibits two relatively distinct periods of egg hatching during the course of the spawning season.

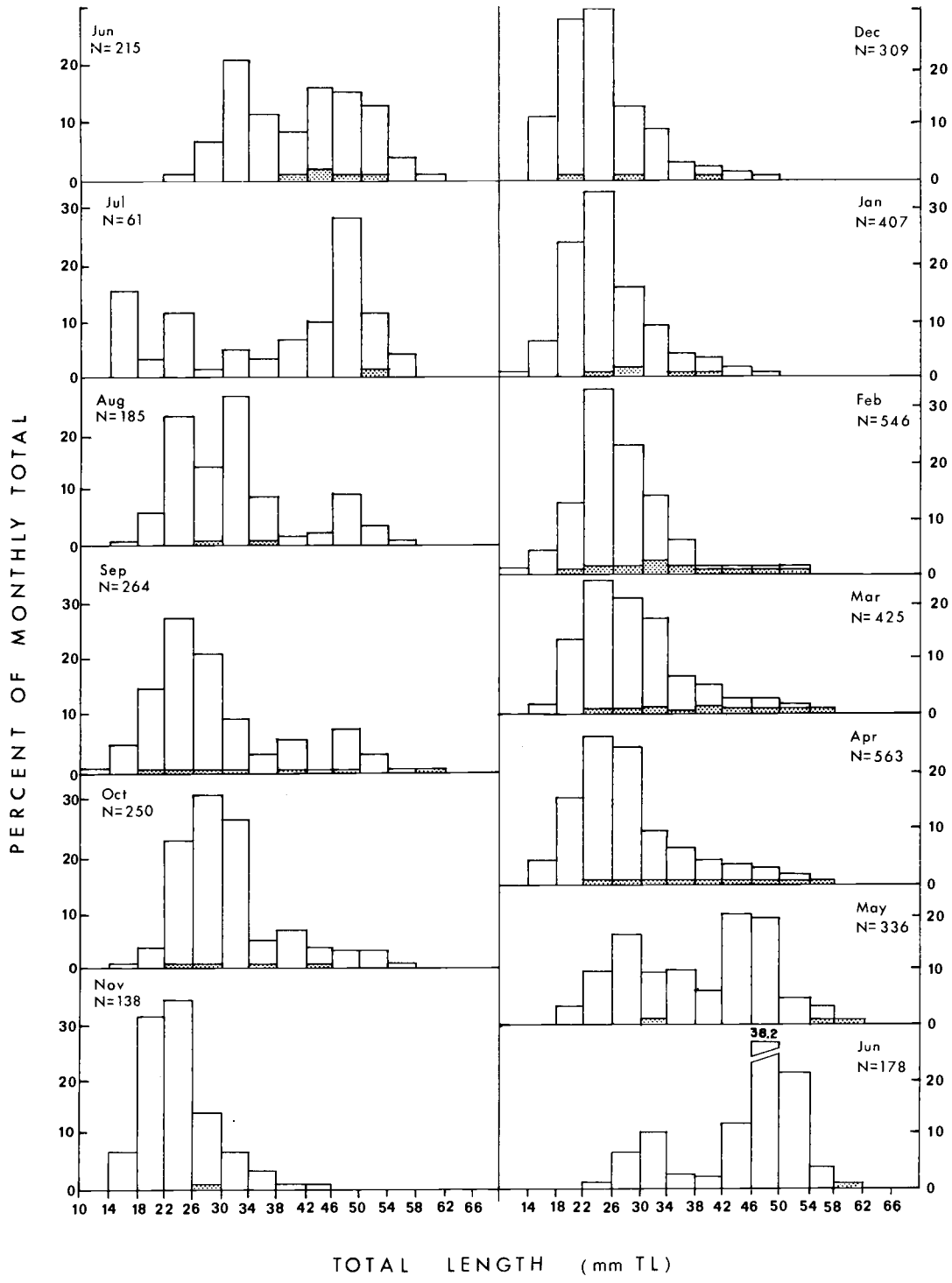


Figure 13. Length-frequency histograms of Crangon nigricauda in Yaquina Bay, Oregon, 1975-76. Clear bars = total animals; shaded bars = infected animals.

Crangon stylirostris

A total of 1,674 C. stylirostris was collected from the Seal Rock sampling station. Apparent movements of these shrimp into and out of the sampling site made the collection of large samples difficult to obtain without repeated efforts each month. Generally, this species was more easily collected during the fall and winter than in the spring and summer. Figure 14 shows the relative proportions of the size classes collected each month. The smallest individuals (13 mm TL) appeared in June, 1976, while the largest (64 mm TL) were collected in May, June, 1976, and July.

Spawning began in December, when 23.1% of 13 mature females collected carried eggs and continued through September when two of seven mature females were gravid. Spawning was at a peak in May, when 40.6% of 69 mature females carried eggs. Juvenile recruitment occurred from May through August with maximum numbers in June, 1976 (Fig. 14).

Prevalence and Intensity of Infection

Data on the prevalence and intensity of pleistophoran infections were pooled by month according to shrimp species and are shown in Table 1. Light infections were difficult to recognize without the aid of a dissecting microscope. Medium and heavy intensity infections

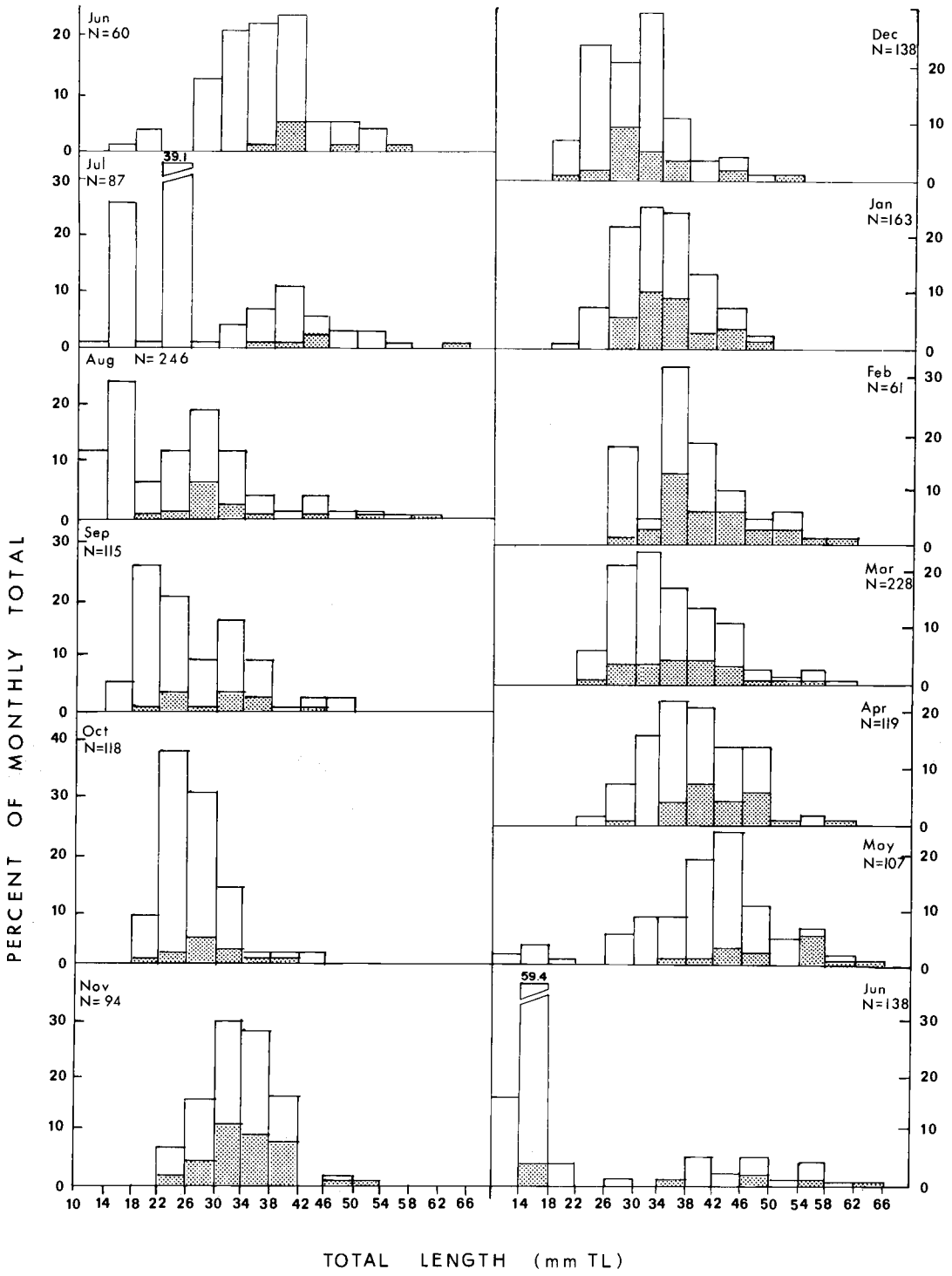


Figure 14. Length-frequency histograms of Crangon stylirostris at Seal Rock, Oregon, 1975-76. Clear bars = total animals; shaded bars = infected animals.

Table 1. Prevalence and intensity of infections of Pleistophora sp. in Crangon spp., 1975-76.

Month	Crangon franciscorum					Crangon nigricauda					Crangon stylirostris				
	No. of shrimp	Prev. (%)	Intensity (%)* L	M	H	No. of shrimp	Prev. (%)	Intensity (%)* L	M	H	No. of shrimp	Prev. (%)	Intensity (%)* L	M	H
June 1975	207	1.0	50	50	-	215	5.1	-	100	-	60	10.0	-	50	50
July	61	0.0	-	-	-	61	1.6	-	-	-	87	5.7	20	40	40
August	80	10.0	100	-	-	185	1.1	100	-	-	246	12.6	84	3	13
September	52	17.3	100	-	-	264	3.0	75	-	25	115	18.3	77	4	19
October	98	10.2	30	60	10	250	1.6	25	50	25	118	14.4	82	6	12
November	142	30.3	60	30	10	138	0.7	100	-	-	94	35.1	48	33	19
December	123	29.3	56	19	25	309	1.3	75	25	-	138	23.9	64	21	15
January 1976	121	17.4	38	33	29	407	2.5	70	30	-	163	30.1	49	35	16
February	245	12.7	23	45	32	546	8.6	60	25	15	61	41.0	20	40	40
March	137	9.5	24	38	38	425	4.2	42	42	16	228	21.5	28	41	31
April	110	2.7	-	-	100	563	2.5	21	50	29	119	22.7	8	48	44
May	76	0.0	-	-	-	336	1.2	25	25	50	107	14.0	7	27	66
June	104	0.0	-	-	-	178	0.6	-	-	100	138	8.7	42	8	50

*L = scattered patches of infection; M = approximately half of tissue infected; H = all tissue appears infected.

were easily observed with the unaided eye. The musculature of shrimp carrying heavy infections was soft and mushy and had a characteristic white opaque appearance in contrast to the firm and translucent tissue of uninfected shrimp.

Crangon franciscorum

Table 1 shows the prevalence of Pleistophora sp. and relative proportions of light, medium, and heavy infections in C. franciscorum samples. Infected shrimp were collected in all months except May, June, 1976, and July. The prevalence of infection was 10% or below from March through August, then increased in the fall, reaching a peak of 30.3% in November before declining during the winter and spring.

Infections of light intensity were first observed in June, 1975, and continued through September when all infected shrimp had light infections (Table 1). As the year progressed, the intensity of infection increased until February through April, when most infected shrimp carried medium or heavy infections. The intensity of infection also increased with the size of the shrimp (Table 2); juvenile shrimp as small as 24 mm TL had light or medium infections, while adults as large as 78 mm TL always had medium or heavy infections. Lightly infected shrimp averaged 38.9 mm TL; shrimp with medium infections averaged 47.1 mm TL; heavily infected shrimp averaged

Table 2. Intensity of infections of Pleistophora sp. in Crangon spp. in relation to size.

Size class (mm TL)	Crangon franciscorum				Crangon nigricauda				Crangon stylirostris			
	No. of shrimp	Intensity (%)*			No. of shrimp	Intensity (%)*			No. of shrimp	Intensity (%)*		
		L	M	H		L	M	H		L	M	H
15-18	0	-	-	-	0	-	-	-	5	100.0	-	-
19-22	0	-	-	-	5	100.0	-	-	5	100.0	-	-
23-26	5	80.0	20.0	-	16	75.0	25.0	-	19	100.0	-	-
27-30	15	86.7	13.3	-	24	83.3	16.7	-	56	87.5	12.5	-
31-34	15	93.3	6.7	-	20	65.0	35.0	-	60	61.7	30.0	8.3
35-38	22	59.0	36.4	4.6	13	30.8	46.2	23.0	60	31.7	55.0	13.3
39-42	20	55.0	25.0	20.0	16	37.5	56.3	6.2	40	12.5	60.0	27.5
43-46	32	50.0	28.1	21.9	12	-	66.7	33.3	32	9.4	21.9	68.7
47-50	21	33.3	47.6	19.1	8	12.5	37.5	50.0	16	-	18.7	81.3
51-54	22	22.7	36.4	40.9	7	-	42.9	57.1	7	-	-	100.0
55-58	7	28.6	28.6	42.8	2	-	50.0	50.0	9	11.1	-	88.9
59-62	11	-	54.5	45.5	4	-	-	100.0	4	-	-	100.0
63-66	2	-	50.0	50.0	0	-	-	-	1	-	-	100.0
67-70	1	-	-	100.0	0	-	-	-	1	-	-	100.0
71-74	2	-	-	100.0	0	-	-	-	0	-	-	-
75-78	1	-	-	100.0	0	-	-	-	0	-	-	-

*L = scattered patches of infection; M = approximately half of tissue infected; H = all tissue appears infected.

53.3 mm TL. All shrimp exceeding 66 mm TL were heavily infected (Fig. 13).

A comparison of the mean size of infected shrimp to that of uninfected shrimp by month is shown in Table 3. Infected shrimp always averaged larger than uninfected shrimp, despite monthly fluctuations in size due to growth and juvenile recruitment.

Crangon nigricauda

The prevalence of Pleistophora sp. in C. nigricauda was always low and varied little throughout the year. The highest prevalence observed was 8.6% in February; in the remaining months it was 5% or lower (Table 1).

Since the numbers of infected shrimp were low, it was difficult to quantify the progression of infection intensity through the year. Generally, light infections predominated during fall and winter and heavier infections in spring and summer. Table 2 gives the data on intensity of infection by size class for C. nigricauda. Intensity of infection increased with shrimp length. Juvenile shrimp with an average length of 29.7 mm TL had light infections; young adults averaging 39.3 mm TL had medium infections, and adults with a mean size of 48.8 mm TL heavy infections. Of the six individuals that exceeded 58.0 mm TL, four were infected.

Table 3. Mean sizes of infected and uninfected Crangon spp., 1975-76.

Month	Crangon franciscorum				Crangon nigricauda				Crangon stylirostris			
	Total N	Mean size (mm TL)	Total inf.N	Mean size (mm TL)	Total N	Mean size (mm TL)	Total inf.N	Mean size (mm TL)	Total N	Mean size (mm TL)	Total inf.N	Mean size (mm TL)
June 1975	207	51.8	2	58.0	215	41.2	11	45.8	60	35.2	66	43.8
July	61	30.4	0	-	61	37.9	1	53.0	85	26.7	5	47.8
August	80	28.9	8	31.1	185	30.3	2	32.0	246	24.7	31	31.6
September	52	39.7	9	42.8	264	27.3	8	36.8	115	25.3	21	30.0
October	98	41.0	10	46.5	250	31.8	4	33.0	118	27.6	17	29.7
November	142	39.0	42	40.1	138	24.4	1	30.0	94	34.0	33	35.3
December	123	34.8	36	37.6	309	25.1	4	27.6	138	29.9	33	31.8
January 1976	121	38.7	21	44.8	407	25.7	10	33.5	163	33.8	49	35.0
February	245	46.3	31	52.8	546	28.1	47	32.9	61	38.8	25	41.6
March	137	45.1	13	48.0	425	30.0	18	38.1	228	35.3	49	37.7
April	110	49.7	3	71.0	563	30.1	14	40.0	119	38.1	27	43.2
May	76	51.3	0	-	336	38.8	4	52.3	107	41.0	15	51.3
June	104	52.6	0	-	178	45.9	1	60.0	138	21.0	12	36.7

Mean lengths of infected and uninfected C. nigricauda are compared by month in Table 3. The mean lengths of infected shrimp were always greater than those of uninfected shrimp.

Crangon stylirostris

The prevalence of pleistophoran infection in C. stylirostris samples is shown in Table 1. It was above 20% from November through April and below 20% from May through October. The highest prevalence observed was 41.0% in February.

Light infections predominated in August, September, and October, and heavy infections in May and June (Table 1). The intensity of infection increased as the length of shrimp increased (Table 2). The mean length of shrimp with light infections was 31.3 mm TL; shrimp with medium infections averaged 36.7 mm TL; heavily infected shrimp had a mean length of 46.5 mm TL. Most large shrimp, including all those over 62 mm TL, were infected (Fig. 15).

A monthly comparison of mean lengths of uninfected and infected shrimp shows that infected shrimp were always larger than uninfected shrimp (Table 3).

Relationship of Microsporidan Infection to Shrimp Fecundity and Sex Ratio

Determination of sex of the crangonids by secondary sex characters was never difficult. Intersexes, if present, were not detected grossly. Gravid female crangonids were always uninfected. Histological sections of gonads of 45 infected and 45 uninfected mature shrimp showed that the ovaries of microsporidan-infected shrimp were always small and never developed beyond Stage 1 (a pair of thread-like tubes containing ova with very little yolk; Meredith, 1952), when compared with those of uninfected shrimp of like size and collected at the same time of year (Figs. 15 and 16). Neither yolk deposition nor ova development occurred in infected shrimp.

Generalizations about the effect of microsporidosis on males are difficult to make, since few infected males were captured. Most infected males collected of each species were small and considered sexually immature. One exception, a 76 mm TL *C. franciscorum* with male secondary sex characteristics, was collected in March, but died, preventing histological examination of the gonads.

The results of comparing the sex ratios of uninfected females to males to those of infected shrimp are shown in Table 4. In all three species of crangonids, the sex ratio of infected shrimp was shifted toward the females.

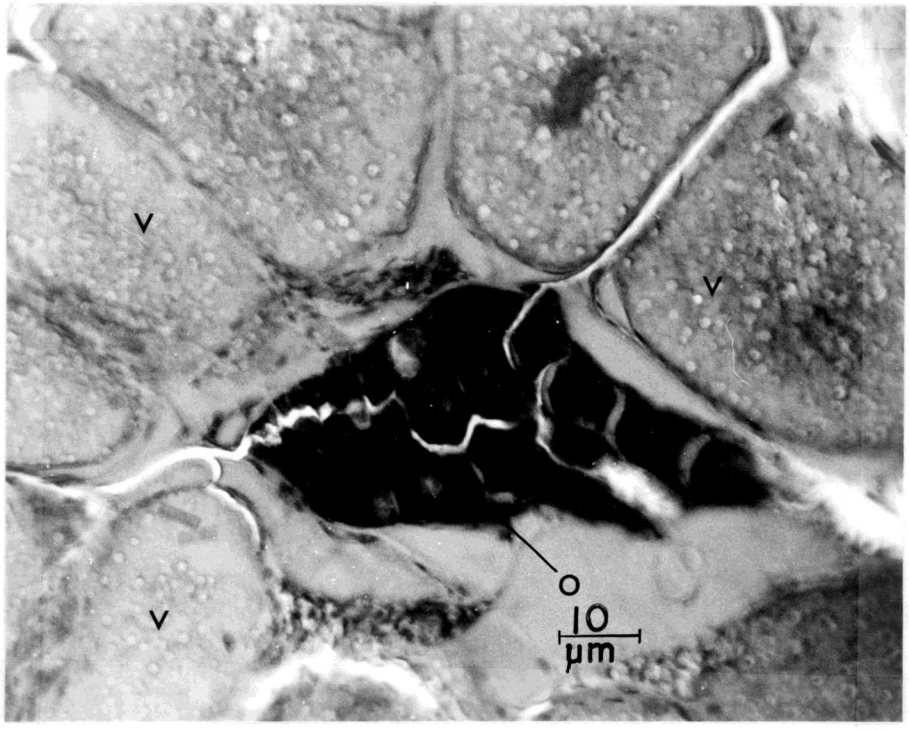


Figure 15. Ovary of uninfected crangonid shrimp, stage 7 (Meredith, 1952). Hematoxylin and eosin. v = yolk granules; o = developing oocytes.

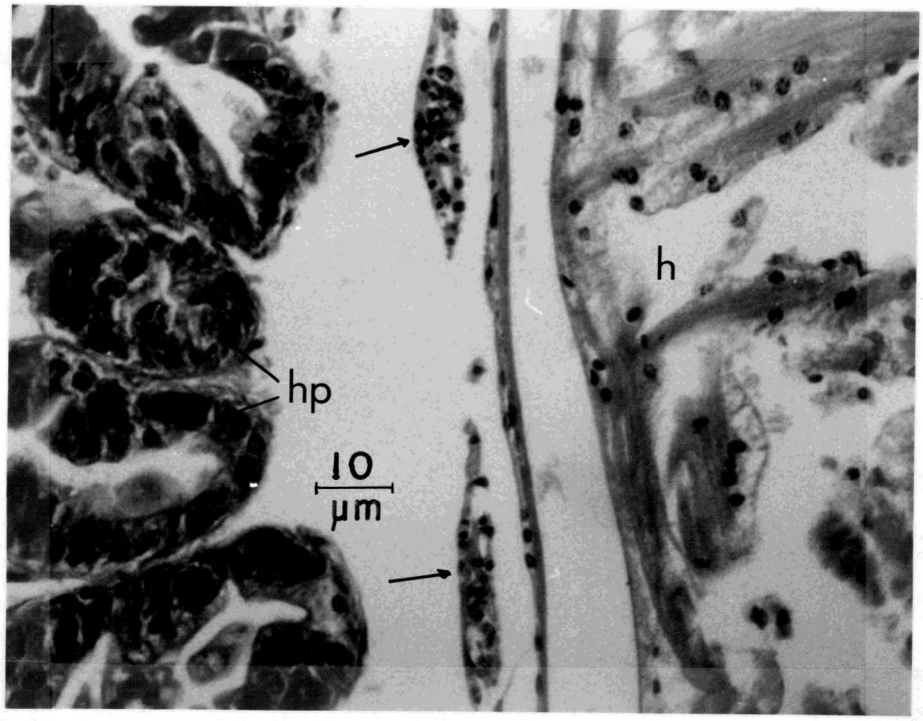


Figure 16. Ovaries of infected crangonid shrimp (arrows), stage 1 (Meredith, 1952). Hematoxylin and eosin. h = heart; hp = hepatopancreas.

Table 4. Sex ratios of Crangon spp.*

	Crangon franciscorum	Crangon nigricauda	Crangon stylirostris
Total N	743:653 1.41:1	2091:1241 1.68:1	963:222 4.34:1
Uninfected shrimp	602:630 1:1.05	1987:1231 1.61:1	715:204 3.5:1
Infected shrimp	141:23 6.13:1	104:10 10.4:1	248:18 13.5:1

*Ratios expressed as females:males.

Shrimp Rearing Experiments

Attempts to rear larvae in 2-gal or 20-gal aquaria by feeding various wild-caught zooplankters and diatoms were unsuccessful. Barnacle larvae were also tried and were too large to be ingested by the early stage larvae.

Attempts to rear crangonid larvae on brine shrimp in 1500 ml beakers met with little success; nine C. franciscorum survived through metamorphosis which occurred from 30-60 days after hatching. Of these nine, two females became infected with a microsporidan parasite. The infections were not noted until the shrimp were 19 and 21 mm TL and five months of age. The means by which they were infected is unknown. One shrimp was fixed in Bouins and sectioned to confirm the identity of the parasite as Pleistophora sp. The other was maintained at room temperature in a 2-gal aquarium

and the rate at which the infection spread within the shrimp was observed until it died at the age of 13 months.

Greater success in rearing crangonids was subsequently obtained by placing groups of eight to ten newly hatched larvae in 400-ml beakers at inflow water temperatures and fed brine shrimp nauplii. Mortality was high (to 90%) during the first ten days in three groups that were not fed during the first 36 hr. Mortality was below 30% in seven groups fed nauplii continuously. Following metamorphosis, the surviving post-larval shrimp were used in infection experiments.

Laboratory Experiments on the Transmission of *Pleistophora* sp.

Six experiments were conducted in an effort to determine the conditions necessary to effect microsporidan transmission to crangonid shrimp. Table 5 outlines the various treatments employed.

Experiment 1. Mature shrimp of each species were allowed to feed on carcasses of naturally infected crangonids. The temperature was maintained at 13C for the duration of the 30-day experiment. No shrimp showed gross signs of infection; histological examination of five experimental and five control shrimp of each species showed negative results.

Experiment 2. To determine the effect of a possible vector on transmission, *Artemia salina* were allowed to feed on fresh spores

Table 5. Laboratory conditions of experiments on the transmission of Pleistophora sp.

Experiment no.	Experimental shrimp species	Spores used	Treatment*	No. of shrimp/ treatment	Holding temp. (C)	No. of days from exposure to termination	Results
1	mature Crangon franciscorum	fresh	a, e	10	13	30	neg
	mature C. nigricauda	fresh	a, e	10	13	30	neg
	mature C. stylirostris	fresh	a, e	10	13	30	neg
2	immature C. nigricauda	fresh	b, e	35	15	70	neg
3	immature C. nigricauda	held at room temp. 1 month	a, b, e	5	20	63	neg
4	mature C. stylirostris	held at room temp. 60 days	b, e	30	15	59	neg
5	juvenile C. stylirostris	held at 6 C 60 days	a, b, c, e	6	16	50	neg
6	post-larval C. stylirostris	fresh	d, e	12	12-17	45	neg

* a, shrimp fed infected shrimp carcasses; b, shrimp fed Artemia salina that had been allowed to ingest spores; c, shrimp fed spores allowed to pass through fish digestive tract; d, shrimp fed Artemia salina that had been allowed to feed on spores from fish feces; e, control, shrimp not exposed.

from C. nigricauda before these brine shrimp were fed to immature (27-33 mm TL) C. nigricauda. The temperature was maintained at 15C, except for one instance 35 days into the experiment when the temperature dropped to 9C due to thermostat failure. No infections were detected grossly in any shrimp, nor histologically in 10 experimental and 10 control shrimp.

Experiment 3. Spores from C. nigricauda were aged for 30 days in bay water at room temperature (20C) before being fed to Artemia salina. The brine shrimp were then fed to immature C. nigricauda (29-32 mm TL) and held at room temperature. After 63 days, no infections were found grossly or histologically in five experimental or five control shrimp.

Experiment 4. To observe the effect of further aging on the infectivity of the spores, Artemia salina were allowed to feed on spores aged for 60 days at room temperature. Temperature was maintained at 15C for 37 days until thermostat failure caused an abrupt rise to 28C after which the temperature was manually controlled between 14C and 19C. After a total of 59 days, no shrimp showed gross signs of infection and histological examinations of 10 control and 10 experimental were negative.

Experiment 5. In the event that younger shrimp are more susceptible to infection, juvenile ($\approx$20 mm TL) C. stylirostris were fed brine shrimp exposed to aged spores, infected carcasses, and

spore-containing sand sole feces. After 50 days, gross and histological examinations of all control and experimental shrimp were negative.

Experiment 6. To further test the susceptibility of differently aged shrimp, post-metamorphic shrimp (≈7 mm TL) raised in the laboratory were fed brine shrimp nauplii that had been fed spore-containing sand sole feces. The temperature fluctuated between 12C and 15C. After 45 days, gross and histological examinations of all control and experimental shrimp were negative.

Spread of Infection within Shrimp

In eight of the nine Crangon stylirostris involved in this experiment, the microsporidan infection was observed to spread within the individual shrimp. Table 6 summarizes the results of the observations made including the initial and final location of the parasite within the host (Fig. 3), and initial and final intensities of infection. In all cases, observations were continued until the shrimp died.

The greatest change was noted in the individuals that were observed for the longest period of time. Shrimp #5 was first observed with a localized light infection in the abdomen. During the 99-day period of observation, the infection spread anteriorly and posteriorly in the abdomen and into the pleopods. The spread of infection in the pleopods proceeded posteriorly, the anterior pairs being infected first, then the next posterior pair until all

Table 6. Observations on spread of infection in Crangon stylirostris.

Shrimp No.	Size (mm TL)	Initial Infection Site & Intensity *				Final Infection Site & Intensity *				Duration (days)
		I	II	III	IV**	I	II	III	IV**	
1	32	40	40	40	-	40	40	40	1	26
2	34	30	-	-	-	30	30	30	1	64
3	29	30	-	-	-	50	30	30	-	10
4	33	30	5	5	-	30	10	10	-	14
5	31	-	25	-	-	35	35	35	1-5	99
6	35	45	45	45	-	75	75	75	1	71
7	32	65	65	65	-	90	90	90	1-5	64
8	32	45	45	45	-	45	45	45	-	4
9	30	50	50	50	-	85	85	85	1-4	64

*Refer to Fig. 3 for site identification: I = first two abdominal segments; II = second two abdominal segments; III = last two abdominal segments; IV = pleopods; intensity in %.

** - = not present; numbers indicate pairs of pleopods infected.

five pairs contained the infection. In shrimps #6, 7, and 9, medium infections were initially observed throughout the abdomen. At the end of the observations, the intensity had increased in each region and had spread into the pleopods. In shrimps #7 and 9 the infection in the pleopods proceeded posteriorly as it did in #5.

In shrimps #2, 3, and 4, infection was fairly localized when first observed. After a variable time period, the infection had spread posteriorly, and in #2 into the first pair of pleopods. The only change observed in shrimp #1 after 26 days was the appearance of infection in the first pair of pleopods. Shrimp #8 died before any change was visible.

In the single infected Crangon franciscorum raised in the laboratory, the infection spread as observed previously in C. stylirostris. The infection was first observed when the shrimp was five months old and when it was light to medium in intensity throughout the abdomen. After 30 days, the infection was of medium intensity and after 90 days, heavy. The shrimp died at an age of 13 months, five months after the infection reached maximum intensity.

The rate of spread of infection appeared to increase with the intensity of infection in all shrimp observed. The increase in intensity from light to medium took approximately 90 days after infections were detected, and from medium to heavy took an additional 60 days. In all cases, infections spread throughout the abdomen by the time the

infection was of medium intensity. Thereafter infections spread to distal areas and increased in intensity.

Effect of Low Oxygen Stress on Infected Shrimp

Generally, the microsporidan-infected shrimp succumbed in the low oxygen stress experiment before the uninfected shrimp. The dissolved oxygen levels in each beaker containing infected shrimp decreased with time for about 12 hr then became stable. The average decrease per 2-hr interval during this time was 0.99 ppm (0.89=sd). Seven of the nine infected shrimp died during the experiment (Table 7), average survival time being 24 hr (14.28=sd). The largest infected shrimp died first, followed by the smaller shrimp. Death of these shrimp came at a mean dissolved oxygen level of 1.72 ppm (0.72=sd), while the dissolved oxygen level in the beakers containing the two surviving shrimp remained constant at approximately 1.7 and 2.0 ppm respectively for 30 hr before termination of the experiment.

Dissolved oxygen levels in the beakers containing uninfected shrimp decreased at an average rate of 0.81 ppm (0.54=sd) per 2-hr interval for the first 12 hr, then became stable. Uninfected shrimp survived for a mean of 34.9 hr (14.0=sd). Four of the nine shrimp died during the experiment at mean dissolved oxygen level of 2.27 ppm (1.91=sd). However, one of these (53 mm TL) died early at a

Table 7. Response of Crangon stylirostris to stress.

Uninfected Shrimp				Infected Shrimp			
Shrimp Size (mm TL)	Hours Duration	Oxygen Content (ppm)	Result	Shrimp Size (mm TL)	Hours Duration	Oxygen Content (ppm)	Result
49	46	2.0	Shrimp alive	48	28	2.1	Shrimp dead
49	46	2.7	Shrimp alive	49	46	2.0	Shrimp alive
49	46	1.7	Shrimp alive	49	46	1.7	Shrimp alive
53	10	5.1	Shrimp dead	50	26	1.1	Shrimp dead
54	28	1.0	Shrimp dead	52	20	1.5	Shrimp dead
55	22	1.8	Shrimp dead	58	20	1.3	Shrimp dead
57	46	1.6	Shrimp alive	64	14	1.3	Shrimp dead
57	46	1.6	Shrimp alive	65	8	1.6	Shrimp dead
63	24	1.2	Shrimp dead	66	8	3.2	Shrimp dead
Control	46	7.4					

relatively high dissolved oxygen level (5.1 ppm). A bopyrid isopod (Argeia pugettensis) had been removed from this shrimp five days before the start of the experiment and may have had a deleterious effect on the shrimp. The other three uninfected shrimp that died did so at dissolved oxygen levels similar to those at which infected shrimp died. The dissolved oxygen levels of the water in which the five shrimp survived remained constant at an average of 1.8 ppm for 28-34 hr before termination of the experiment.

A thin layer of spores was found on the bottom of the beakers containing the two largest infected shrimp 6 hr before they died, although they remained intact.

Histopathology

Pleistophora sp. occurred only in areas of striated, skeletal musculature, the abdominal muscles being the most common site of infection. In heavily infected Crangon spp., the infection was also present in distal areas such as the periopods, pleopods, uropods, and antennae. Examination of sectioned material showed that the muscle was the only tissue parasitized. In no instance were gills, hepatopancreas, gonads, or cardiac muscle infected.

Parasitized muscles were lysed and replaced by microsporidan sporonts, sporoblasts, and spores (Fig. 4). Contiguous muscle fibers, although uninfected, were often contorted and twisted around

the infected areas (Fig. 17). Necrotic muscle fibers, characterized by poor staining and deterioration of myofibrils and striations, but not containing spores, were occasionally observed in areas where the infections were intense (Fig. 18).

In lightly infected shrimp, the infections were concentrated in small areas scattered throughout the musculature (Fig. 19). When viewed in cross section, it was apparent that muscle bundles were not completely replaced; mysia surrounded muscle bundles and groups of cysts. In more intense infections, the areas of infection were larger and more numerous and in heavily infected shrimp, the mysia enclosed masses of cysts, muscle bundles being completely eliminated. In large areas of intense infection, the pansporoblastic membrane had often broken and free spores were observed throughout the region. Rarely, and only in heavily infected shrimp, individual or small numbers of cysts were enclosed by nucleated cells (Fig. 20). It is possible that this is the result of an attempt by the host's hemocytes to wall off the parasite.

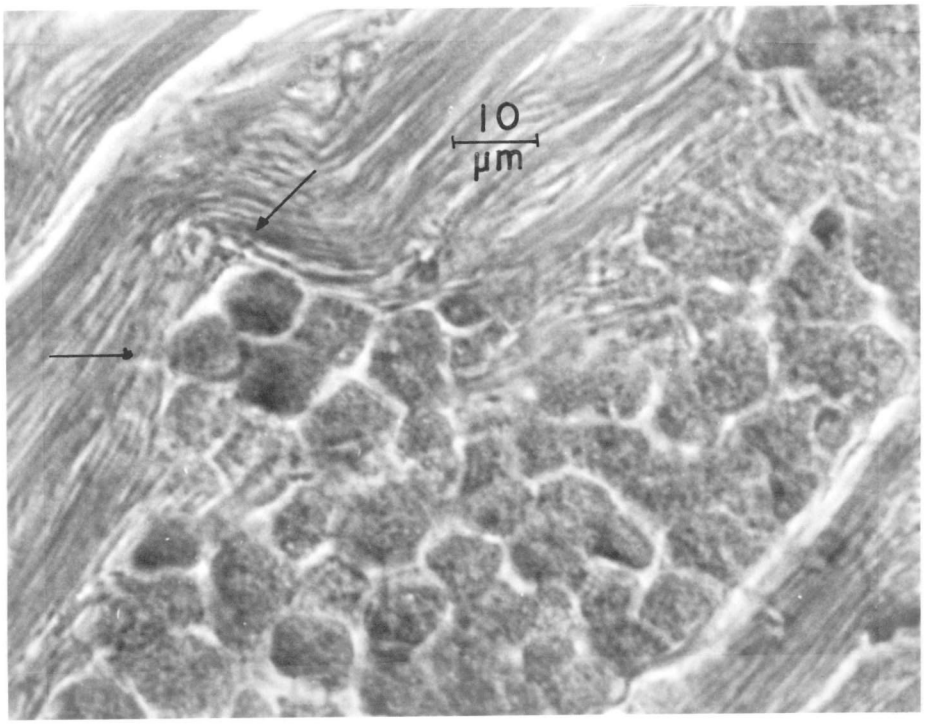


Figure 17. Photomicrograph of infected muscle, showing uninfected adjacent tissue. Note displacement of uninfected fibers (arrows). Hematoxylin and eosin.

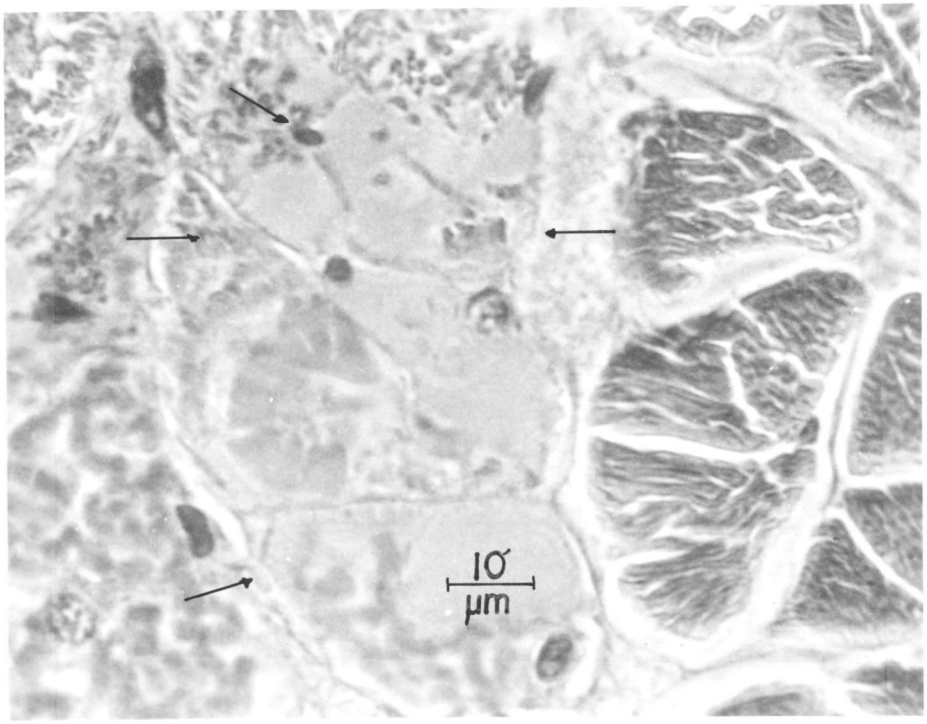


Figure 18. Photomicrograph of necrotic muscle (enclosed by arrows). Hematoxylin and eosin.

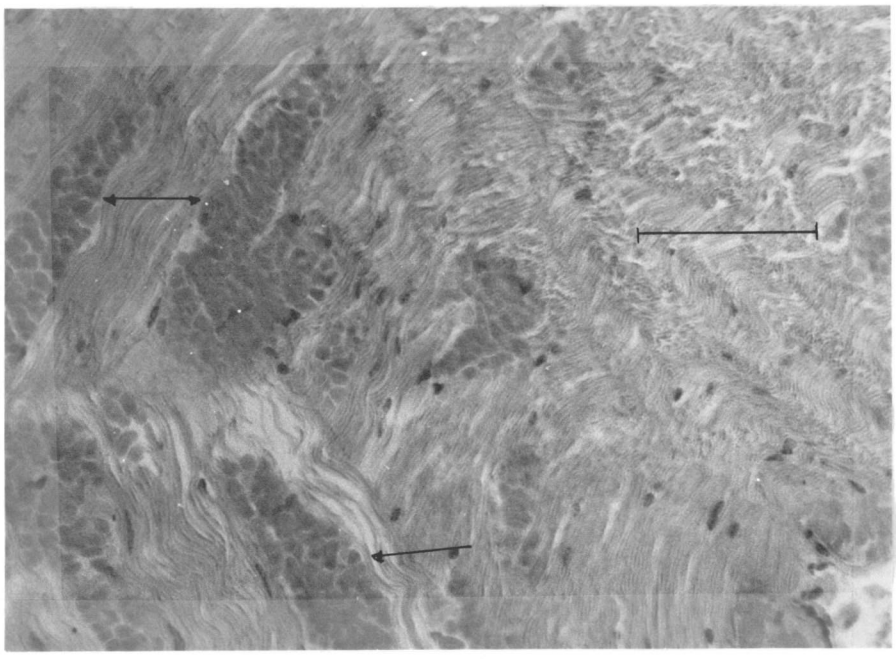


Figure 19. Photomicrograph of lightly infected musculature. Patches of infection indicated by arrows. Scale = 100 μm.

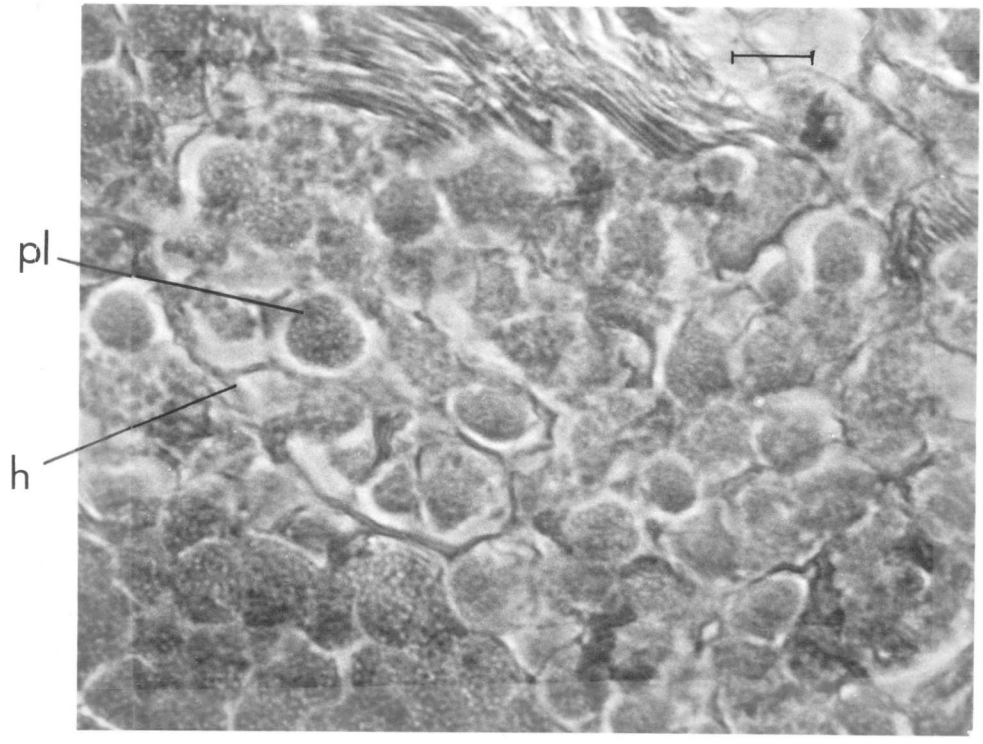


Figure 20. Pleistophora sp. cysts enclosed by host hemocytes. pl = cysts; h = hemocytes. Scale = 10 μm.

DISCUSSION

The life cycle of Pleistophora sp. was not demonstrated experimentally in this study. It may follow a pattern similar to that of Nosema sp. in the blue crab as follows: spores are ingested by the host and the sporoplasm passes through the extruded polar filament to hemocytes in the submucosa below the gut epithelium where schizogony begins. The trophozoites are then transported via hemocytes in the hemolymph to muscle proximal to the hemocoel, sporogenesis occurring in the final target tissue (Weidner, 1970, 1972). The sporogonic divisions and resultant increase in spore numbers within the muscle sarcoplasm most likely cause host cell hypertrophy and subsequent lysis of the fibers.

The nucleated cells surrounding small groups of parasite cysts were probably hemocytes, an apparent attempt by the host to wall off the foreign material. Lightner (1975) reported that hyphae of the fungus Fusarium spp. are encapsulated by hemocytes of penaeid shrimp. Gammarids infected with Nosema spp. or Thelohania sp. secrete a chitinoid substance around necrotic muscle tissue and destroyed spores (Pixell-Goodrich, 1929), forming a nodule, but no such secretions were observed here.

Laboratory observations have demonstrated that the infection spreads within the host without continuous exposure to spores. Since

the intestinal cells were never observed to be parasitized, it appears that parasite multiplication following initial infection results from repetitive shizogonic divisions in the musculature. On the basis of observations of microsporidosis in fishes, Lom (1970) has suggested that vegatative stages continue to multiply following the completion of initial schizogonic and sporogonic stages. The small bi-, tri-, or quadrinucleate cells observed at the edges of areas of infection (Fig. 5) are probably the result of second or third generation schizogonic divisions. Spore germination may also occur within the host (Lom, 1970), although no evidence of such an event, i.e. empty spores, was observed during this study.

Unsuccessful attempts to effect experimental transmission here and elsewhere (Roth and Iversen, 1971) indicate that the conditions under which the spores become infective are critical. Several factors may contribute to the infectivity of the spores: age of the spores, age of the shrimp, temperature (McVicar, 1975; Olson, 1976), or the necessity of an intermediate vector (Roth and Iversen, 1971; Iversen and Kelly, 1976). Iversen and Kelly (1976) recently succeeded in infecting a penaeid shrimp with Thelohania duorarum by allowing the spores to pass through a fish's digestive tract before feeding to shrimp. Since they were able to infect post larval, but not juvenile shrimp by this method, it is probable that the age of the shrimp is the more critical factor.

The spawning seasons, periods of juvenile recruitement, and sex ratios of Crangon franciscorum and C. nigricauda observed here are similar to those observed by Krygier and Horton (1975). The slight differences in spawning season and recruitment observed here were probably due to differences in sampling methods and to variations in the timing of seasonal migrations influenced by changes in temperature and salinity. The biology of C. stylirostris has not been previously studied but the spawning season and recruitment periods observed are similar to those of the other two crangonid species. Seasonal movements of crangonids subject to temperature and salinity changes were previously observed in Britain (Lloyd and Yonge, 1947; Meredith, 1952; Allen, 1966), Chesapeake Bay (Haefner, 1976), San Francisco Bay (Israel, 1936), and Yaquina Bay (Krygier and Horton, 1975). Krygier and Horton (1975) also report that C. franciscorum and C. nigricauda undergo a spawning migration from Yaquina Bay to deeper water in the summer, explaining the low abundance of these shrimp during that period. The same is probably true of C. stylirostris.

Studies on the prevalence and seasonality of microsporidan infections are few. Of the infected penaeid shrimp collected by Constransitch (1970), 6.5% hosted Pleistophora sp. infections and 93.5% Nosema sp. or Thelohania spp. infections, but the prevalence in the shrimp population was not determined. Villela, Iversen, and

Sindermann (1970) reported that Thelohania duorarum infected 8% of Penaeus duorarum in the wild, but not in culture pond shrimp. Miglarese and Shealy (1974) observed that unidentified microsporidans infected 89.5% of Penaeus setiferus collected in South Carolina in winter and 15% in July.

The reason for the low prevalence of Pleistophora sp. in C. nigricauda when compared to the prevalences in the other species studied is unclear. According to Krygier and Horton (1975), juveniles of C. nigricauda respond differently to temperature and salinity than do the adults and consequently have a different distribution in Yaquina Bay. A possible explanation for the low levels of infection observed in C. nigricauda may be that the susceptible juvenile shrimp do not have access to spores released by dead or dying adults due to this difference in distributional patterns of juveniles and adults. Conversely, C. franciscorum juveniles and adults have similar distributional patterns (Krygier and Horton, 1975) and spores released by infected adults would therefore be readily available to juveniles. This could explain the higher prevalence of infection in this species. The same may also be true of C. stylirostris, since juveniles and adults were captured together within the sampling area.

The rise in prevalences of infection in C. franciscorum and C. stylirostris during the late summer and fall indicates that increasing numbers of shrimp become infected at this time, but not

later when either spores are no longer available or shrimp are not susceptible. The decline in the prevalences of infection observed during the winter and spring may have been due to the death of heavily infected shrimp because most infected shrimp carried medium to heavy infections at this time. Although the lethality of Pleistophora sp. has not been fully documented in the laboratory, the stress experiment using uninfected and infected C. stylirostris demonstrated that infected shrimp succumb more quickly than do uninfected ones. Overstreet (1973) and Bulnheim (1975) suggest that microsporidan infections cause reduced resistance to stress, but do not influence respiratory rate (Bulnheim, 1975; Steven Scarborough, personal communication). Survival of two infected shrimp for an extended period at low oxygen levels in this study remains unexplained, but may be related to the small size of these individuals.

The relationship between intensity of infection and the size of the crangonid shrimp was one of increasing intensity with increasing size. This is in contrast to pleistophoran infections in penaeid shrimp where no such relationship has been found (Constransitch, 1970). The crangonid shrimp are apparently exposed to parasite spores as juveniles during a relatively short period in the summer when the spores have been released by the death or deterioration of heavily infected shrimp. The one- or two-month period between the reduction in numbers of heavily infected adult shrimp and the appearance of

lightly infected juveniles most likely represents the time period required for priming of the spores and for development of the parasite to the stage when it becomes visible in the host.

Parasitic castration was indicated by the absence of gravid infected females. The phenomenon of parasitic castration has been reviewed by Reinhard (1956) and discussed by Charniaux-Cotton (1960). Microsporidan-caused castration, as observed here, also occurs in the shrimp Penaeus setiferus, the causative agent being Thelohania penaei (Overstreet, 1973). It is most likely that oogenesis does not proceed in parasitized Crangon spp. females due either to energy drain or to hormonal imbalance or both.

The observed deviations from the normal sex ratios in the three crangonid species indicate that the microsporidan may interfere with sex determination in the host. The androgenic gland, the source of male sex hormone in malacostracans, controls the differentiation and expression of male sex characters (Carlisle and Knowles, 1959; Charniaux-Cotton, 1960). In the absence of the gland, the gonads develop into ovaries. Interference with this gland or its secretions may cause sex reversal from male to female. Such a phenomenon was observed in gammarid amphipods infected with Octosporea effeminans (Bulnheim, 1975). Rigdon, Baxter, and Benton (1975) noticed a possible microsporidan-caused effect on sex determination when they found an infected hermaphroditic Penaeus setiferus, a

species of shrimp normally dioecious. Microsporidan influence on hormonal regulation in larval flour beetles, Tribolium sp., was shown by Fisher and Sanborn (1964) who demonstrated that a spore extract from Nosema sp. prevented pupation of the host. No such investigations have been made on Pleistophora spp.

Possible explanations for the larger size of the infected shrimp include an accelerated growth rate or a longer life span. It seems most likely that energy not devoted to reproduction in these castrated shrimp is instead utilized to enhance growth.

Coupled with the growing interest in the commercial culture of shrimp and crabs is the importance of the development of methods for diagnosis, prevention, and treatment of disease. It is hoped that the information gained from this study on microsporidosis in crangonid shrimp will prove valuable and give an insight into similar infections in other decapods.

LITERATURE CITED

Allen, J.A. 1966. The dynamics and interrelationships of mixed populations of Caridea found off north-eastern coast of England. Pages 45-66. In Barnes, H. (ed.). Some contemporary studies in marine science. George Allen and Unwin Ltd. London. 716 p.

Balbiani, E.G. 1882. Sur les Microsporides ou Psorospermies des Articules. C.R. Acad. Sci. 95:1168-71.

Baxter, K.N., R.H. Rigdon, and C. Hanna. 1970. Pleistophora sp. (Microsporidia: Nosematidae): a new parasite of shrimp. J. Invert. Pathol. 16:289-91.

Bulnheim, H.P. 1975. Microsporidian infections of amphipods with special reference to host-parasite relationships: a review. Mar. Fish. Rev. 37:39-45.

Carlisle, D.B., and F. Knowles. 1959. Endocrine control in crustaceans. Pages 95-101. Cambridge University Press. Cambridge. 119 p.

Charniaux-Cotton, H. 1960. Sex determination. Pages 411-447. In Waterman, T.H. (ed.). The physiology of Crustacea. Vol. 1. Academic Press. New York. 670 p.

Constransitch, 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. 35 p.

Corliss, J.O., and N.D. Levine. 1963. Establishment of the Microsporidea as a class in the protozoan subphylum Cnidospora. (Abstr.) J. Protozool. 10(Suppl.):26-7.

Fisher, F. Jr., and R.C. Sanborn. 1964. Nosema as a source of juvenile hormone in parasitized insects. Biol. Bull. 126:235-52.

Haefner, P.A., Jr. 1976. Seasonal distribution and abundance of sand shrimp Crangon septemspinosa in the York River-Chesapeake Bay estuary. Chesapeake Sci. 17:131-4.

Haertel, L., and C. Osterberg. 1966. Ecology of zooplankton, benthos, and fishes in the Columbia River estuary. Ecology 48:459-72.

Henneguy, F., and P. Thelohan. 1893. On a sporozoan parasitic in the muscles of the crayfish. Ann. Nat. Hist. 11:195-6.

Holthius, L.B. 1951. Proposed use of the plenary powers to validate the generic name "Crangon" Fabricius, 1798, for the common shrimp and the generic name "Alpheus" Fabricius, 1798, for the snapping shrimps (Class Crustacea Order Decapoda). Bull. Zool. Nomenc. 2:69-72.

Honigberg, B.M., W. Balamuth, E.C. Bovee, J.O. Corliss, M. Gojdics, R.P. Hall, R.R. Kudo, N.D. Levine, A.R. Loeblich, J. Weiser, and D.H. Wenrich. 1964. A revised classification of the phylum Protozoa. J. Protozool. 11:7-20.

Hublou, W.F. 1963. Oregon pellets. Prog. Fish Cult. 25:175-80.

Humason, G.L. 1967. Animal tissue techniques. Pages 303-311. W.H. Freeman and Co. San Francisco. 468 p.

Israel, H.B. 1936. A contribution toward the life histories of two California shrimps, Crago franciscorum (Stimpson) and Crago nigricauda (Stimpson). Calif. Div. Fish. Game, Fish Bull. No. 46. 28 p.

Iversen, E.S., and J.F. Kelly. 1976. Microsporidosis successfully transmitted experimentally in pink shrimp. J. Invert. Pathol. 27:407-8.

Krygier, E., and H.H. Horton. 1975. Distribution, reproduction, and growth of Crangon nigricauda and Crangon franciscorum in Yaquina Bay, Oregon. Northwest Sci. 49:216-40.

Kudo, R. 1924. A biologic and taxonomic study of the microsporidia. Ill. Biol. Monogr. 9:79-344.

Levine, N.D. 1970. Taxonomy of the Sporozoa. J. Parasitol. 56(4), Sect. II, Part 1:208-9.

Lightner, D.V. 1975. Some potentially serious disease problems in the culture of penaeid shrimp in North America. Proc. 3rd U.S.-Japan Meeting on Aquaculture, Tokyo, 1974:75-97.

Lloyd, A.J., and C.M. Yonge. 1947. The biology of Crangon vulgaris in the British Channel and Severn estuary. J. Mar. Biol. Assoc. U.K. 26:626-61.

Lom, J. 1970. Protozoa causing diseases in marine fishes. Pages 101-23. In Sneiszko, S. (ed.). A symposium on diseases of fishes and shellfishes. Amer. Fish Soc. Spec. Publ. No. 5. Washington, D.C. 526 p.

McVicar, A.H. 1975. Infection of plaice Pleuronectes platessa L. with Glugea (Nosema) stephani (Hagenmuller 1899) (Protozoa: Microsporidia) in a fish farm and under experimental conditions. J. Fish Biol. 7:611-19.

Meredith, S.S. 1952. A study of Crangon vulgaris in Liverpool Bay area. Proc. Trans. Liverpool Biol. Soc. 1950-1952:72-109.

Miglarese, J.V., and M.H. Shealy. 1974. Incidence of microsporidean and trypanorhynch cestodes in white shrimp, Penaeus setiferus Linnaeus, in South Carolina estuaries. South Carolina Acad. Sci. Bull. 36:93.

Olson, R.E. 1976. Laboratory and field studies on Glugea stephani (Hagenmuller), a microsporidan parasite of pleuronectid flatfishes. J. Protozool. 23:158-64.

Overstreet, R.M. 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture 2:105-40.

Pixell-Goodrich, H. 1929. Reactions of Gammarus to injury and disease, with notes on some microsporidial and fungoid diseases. Q.L. Micros. Sci. 72:325-53.

Reinhard, E.G. 1956. Parasitic castration of Crustacea. Parasitol. 5:79-107.

Rigdon, R.H., K.N. Baxter, and R.C. Benton. 1975. Hermaphroditic white shrimp, Penaeus setiferus, parasitized by Thelohania sp. Trans. Amer. Fish. Soc. 104:292-5.

Roth, J.N., and E.S. Iversen. 1971. Attempts to transmit experimentally the microsporidian Thelohania duorara, parasitizing the pink shrimp, Penaeus duorarum. Trans. Amer. Fish. Soc. 100:369-70.

Sprague, V. 1966. Two new species of Plistophora (Microsporida: Nosematidae) in decapods, with particular reference to one in the blue crab. J. Protozool. 13:196-9.

_____. 1969. Need for drastic revision of the classification of subphylum Amoebogena. Progr. in Protozool. III Internat. Cong. Protozool. p. 372.

Street, D.A., and V. Sprague. 1974. A new species of Pleistophora (Microsporida: Pleistophoridae) parasitic in the shrimp Palaemonetes pugio. J. Invert. Pathol. 23:153-6.

Villela, J.B., E.S. Iversen, and C.J. Sindermann. 1970. Comparison of the parasites of pond-reared and wild pink shrimp (Penaeus duorarum Burkenroad) in South Florida. Trans. Amer. Fish. Soc. 99:789-94.

Weidner, E. 1970. Ultrastructural study of microsporidian development. I. Nosema sp. Sprague, 1965 in Callinectes sapidus Rathbun. Z. Zellforsch. 105:33-54.

_____. 1972. Ultrastructural study of microsporidian invasion into cells. Z. Parasitenkunde 40:227-42.