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A Survey of Potential Disease-Causing

Organisms in Bait Shrimp from



U. S. DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration

National Marine Fisheries Service

Southeast Fisheries Center Galveston, Laboratory Galveston, Texas 77550

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West Galveston Bay, Texas

ΒY

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A SURVEY OF POTENTIAL DISEASE-CAUSING ORGANISMS IN BAIT SHRIMP FROM WEST GALVESTON BAY, TEXAS

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A SURVEY OF POTENTIAL DISEASE-CAUSING ORGANISMS IN BAIT SHRIMP FROM WEST GALVESTON BAY, TEXAS

ABSTRACT

Data are presented from a one year survey of potential disease-causing organisms in white shrimp, <u>Penaeus setiferus</u>, and brown shrimp, <u>P. aztecus</u>, collected by commercial bait dealers in West Galveston Bay, Texas. The shrimp examined were purchased from bait dealers at 12 to 18 day intervals beginning on November 19, 1973 and continuing until November 22, 1974.

Bacteria isolated from the blood of shrimp were predominately of the genera <u>Vibrio</u>, <u>Aeromonas</u>, and <u>Pseudomonas</u>. <u>Vibrio</u> occurred most often during July, August, and September, <u>Aeromonas</u> in the spring and fall, and, <u>Pseudomonas</u> in September, October, and November. One imperfect fungus of the genus Fusarium was isolated in March.

An unidentified suctorian was observed attached to shrimp gills from May through December, while the peritrichous ciliate, <u>Lagenophrys</u>, was on the gills during all months except August and was most frequent in May and June. Two species of the stalked, peritrichous ciliates <u>Epistylis</u> and <u>Zoothamnium</u> were observed attached to the gills. <u>Epistylis</u> occurred in March and September, while <u>Zoothamnium</u> was widespread, occurring in every month with the highest incidence in May, June, and July.

The gregarine, <u>Nematopsis</u> <u>penaeus</u>, was observed in the digestive tract of shrimp throughout the study. Only two shrimp exhibiting microsporidosis were found: Nosema nelsoni in April and Thelohania sp. in May.

Larval nematodes were found in 10 to 100% of all samples except in November 1973. Seasonally, the incidence was highest during January through March. Numerous unidentified cestode procercoid larvae were observed in the midgut. The highest incidence occurred in August and September.

The incidence of the larval Trypanorhynchid cestode, <u>Prochristianella</u> <u>hispida</u>, remained fairly constant with little difference occurring between brown and white shrimp. Several unidentified Cyclophylidean larvae were observed encysted in the wall of the midgut of one shrimp and a metacercaria of <u>Opeocoeloides fimbriatus</u> was observed in the hepatopancreas of brown shrimp.

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SURVEY OF POTENTIAL DISEASE-CAUSING ORGANISMS IN BAIT SHRIMP FROM WEST GALVESTON BAY, TEXAS

INTRODUCTION

The objectives of the survey reported herein were to document the incidence, seasonal abundance, and recognition of the major parasites and diseases of white shrimp, <u>Penaeus setiferus</u> and brown shrimp <u>P. aztecus</u>, from West Galveston Bay, Texas. Species utilized in this one-year (Nov. 1973-Nov. 1974) survey were obtained from commercial bait dealers. The resulting data and figures should aid in the recognition and diagnosis of pathological conditions encountered in other natural shrimp populations. All penaeid shrimp morphological terms used follow those presented by Young (1959).

Contributions of diseases to the mortalities of shrimp in natural populations currently are poorly understood. Additionally, helminth and protozoan parasites occur in natural populations of shrimp and may contribute to low survival of juvenile shrimp in estuarine areas. Excellent surveys and reviews of diseases and parasites of penaeid shrimp have been presented by Couch (1978) and Overstreet (1978). The majority of their work, however, was done on shrimp from areas in the northeastern Gulf of Mexico. Further, Johnson (1978) reported his observations in a handbook of shrimp diseases, and Sindermann (1970) has documented a synopsis of principal diseases of marine fish and shellfish.

In nature, diseases probably weaken some shrimp making them easy prey of their many enemies (Neal, 1974). Currently, five major diseases are recognized in penaeid shrimp (Lightner, 1975): (1) mycosis of larval shrimp caused by a <u>Lagenidium</u> sp.; (2) mycotic infection of juvenile shrimp with <u>Fusarium</u> spp.; (3) bacterial infection caused by <u>Vibrio</u> spp. and <u>Benekea</u> spp.; (4) a complex of several gill diseases the causes of which individually or collectively result in respiratory failure; and (5) the "cotton" shrimp group of diseases caused by several species of Microsporida.

MATERIALS AND METHODS

Live shrimp were purchased from commercial bait dealers at 12 to 18 day intervals. All shrimp were collected by trawl from West Galveston Bay waters within easy access to the bait camps. At the time of purchase, the water temperature at a given bait camp was taken with a stem thermometer, and the salinity with a hand-held refractometer (Table 1). The live shrimp were placed in an ice chest, and upon arrival at the laboratory 10 animals were removed for examination; the remainder of the shrimp were maintained in a 1,000-liter holding tank. All shrimp taken for examination were measured (total length, tip of rostrum to tip of telson) and sexed before being processed (Table 1).

There were eight tissue groups observed: (1) blood, (2) gills, (3) hindgut and gland, (4) midgut, (5) foregut, (6) dorsal gland, (7) hepato-pancreas, and (8) gross examination of musculature, heart, and appendages.

Blood samples (ranging in volume from 0.1 cc to 0.5 cc) were drawn with a tuberculin syringe directly from the heart after first swabbing the cuticle with 70% alcohol. The blood was then placed on the surface of plates of tryptic soy agar containing 2.5% NaCl and incubated 24 hr at 28°C. All transfers and isolations were done using the same media. Pure cultures of bacteria were inoculated into slant tubes containing tryptic soy agar containing 2.5% NaCl and sent to D. H. Lewis (Texas A&M University, College of Veterinary Medicine, Department of Microbiology, College Station, Texas) for identification. All biochemical characterizations presented herein follow those presented by Lewis (1973) for aerobic bacteria of fish and shellfish. Routine antibiotic sensitivities were performed by Dr. Lewis on all bacterial isolates made during this study.

The gills were examined by first removing the right branchiostegite with scissors and then excising the gill processes with forceps. A wet mount of the gill tissue was made using sterile seawater and examined microscopically. The exoskeleton and musculature overlying the digestive tract was removed by making two lateral incisions with scissors and lifting the tissue away with forceps. The gut was then removed intact and placed

in sterile seawater in a glass petri dish. The hindgut gland, hindgut, and dorsal gland were placed on individual glass slides with seawater and flattened with a glass cover slip. The midgut and foregut were handled similarly, but were stripped with the edge of the coverslip to remove all fecal material and parasites.

A preliminary gross examination was made of heart, musculature, and appendages for clinical signs of disease. The hepatopancreas was examined using procedures described by Aldrich (1965). The connective tissue sheath covering the hepatopancreas plus some additional hepatopancreatic tissue were examined microscopically.

Tissue specimens were periodically taken from bait shrimp and prepared for histological examination. The tissue specimens were fixed in 10% phosphate buffered formalin and prepared routinely for sectioning. The tissue sections, cut at 7-8 μ , were stained with Harris's hematoxylin and counterstained with eosin. All micrographs of fresh whole mounts and prepared histological sections were made with a Zeiss Photomicroscope II¹/.

Tissue smears were made from shrimp with clinical signs of microsporidian infection and stained with Giemsa stain. Parasites were identified following observations previously published by Overstreet (1973), Sprague and Couch (1971), Corkern (1970), Kruse (1959), and Hutton et al. (1959).

RESULTS

A. Bacteria

There were 17 species of bacteria represented in the isolates, predominantly the genera <u>Vibrio</u> (42.3%), <u>Aeromonas</u> (23.6%), and <u>Pseudomonas</u> (22.8%). Cultures of <u>Lucibacterium harveyi</u>, <u>Gaffyka homari</u>, <u>Chondrococcus</u> columneris, Bacillus sp., and Enterobacter sp. also occurred.

<u>1</u>/Mention of trade names or commercial products does not constitute endorsement by the National Marine Fisheries Service, NOAA.

1. Vibrio

The species of <u>Vibrio</u> isolated during this study were <u>V</u>. <u>alginoly-</u> <u>ticus</u>, <u>V</u>. <u>anguillarum</u>, <u>V</u>. <u>algosus</u>, and <u>V</u>. <u>parahaemolyticus</u>. All four species of <u>Vibrio</u> were positive for indole production and were swarmers on the medium used for isolation (tryptic soy agar containing 2.5% NaCl). Some isolates of <u>V</u>. <u>anguillarum</u> had an acidic reaction with lactose while other species were alkaline. Some species were positive in the Voges-Proskaur test while other species were negative, and some isolates were phosphorescent and others not. The antibiotic sensitivity of <u>V</u>. <u>alginoly</u>ticus and <u>V</u>. anguillarum is shown in Table 2.

The percentage of shrimp from which <u>Vibrio</u> spp. were isolated per month is graphically represented in Figure 1. There were 57 isolates of <u>Vibrio</u> spp. (often more than one <u>Vibrio</u> sp. was isolated per animal) of which 44% were made during July, August, and September.

2. Aeromonas

There were only two species of <u>Aeromonas</u> isolated from bait shrimp: <u>A. shigelloides</u> and <u>A. formicans</u>. <u>A. shigelloides</u> was separated from <u>A. formicans</u> by non-utilization of gelatin. <u>A. formicans</u> was charaterized by production of gelatinase, non-aerogenic, and did not form acetylmethylcarboniol from dextrose (Voges-Proskaur test). The antibiotic sensitivity of the two species is shown in Table 2.

The occurrence of <u>Aeromonas</u> spp. by months is shown in Figure 1. The two species of <u>Aeromonas</u> occurred predominately in the spring and fall and both species were isolated from white shrimp but only <u>A. shigelloides</u> was isolated from brown shrimp.

3. Pseudomonas

Six species of <u>Pseudomonas</u> spp. were isolated from bait shrimp: <u>P. piscicida, P. dentrificans, P. stutzeri, P. fluorescens, P. alcalige-</u> <u>nes</u>, and <u>P. diminuta</u>. The six species produced a wide variety of biochemical reactions, and the antibiotic sensitivity of two of the more common species is shown in Table 2.

The monthly occurrence of <u>Pseudomonas</u> spp. is shown in Figure 1. Pseudomonas spp. were isolated primarily in the fall with approximately 48%

of all isolations made in September, October, and November. The species of <u>P. dentrificans, P. fluorescens</u>, and <u>P. alcaligenes</u> were isolated only from white shrimp, while <u>P. diminuta</u> was isolated only from brown shrimp, and <u>P. piscicida</u> and <u>P. stutzeri</u> were isolated from both shrimp.

B. Fungi

The only fungus isolated during this study was an imperfect fungus of the genus <u>Fusarium</u>. This <u>Fusarium</u> sp. occurred on the gills of one white shrimp in March 1973 and was associated with numerous ciliates and an unidentified filamentous organism (Fig. 2).

The affected white shrimp showed gross clinical signs of black gill disease (Lightner et al., 1976). The <u>Fusarium</u> was isolated in Cantino PYG broth supplemented with 2% NaCl and treated with penicillin and streptomycin to inhibit bacterial growth. The culture was maintained on Sabouraud dextrose agar supplemented with 2% NaCl and with shrimp homogenate (SSS medium - Lightner and Fontaine, 1975). The <u>Fusarium</u> sp. produced micro- and macro-conidia both in artificial media and in shrimp tissues.

C. Protozoa

1. Suctoria

One species of suctorian was observed attached to the gills of bait shrimp (Fig. 3). The organism was attached to the gills by a disk, with a stout stalk, lorica, and suctorial and penhensile tentacles present. Unconfirmed tentative taxonomic descriptions (Kudo, 1954) place the organism in the family Ephelotidae and the genus Ephelota.

The monthly incidence of this organism is presented in Figure 4. The suctorian was not observed on the gills of shrimp from January through April 1974. Microscopic examinations of fresh whole mounts of affected gill tissue indicated that no histological damage was caused by the suctorian.

2. Ciliata

a. Peritricha

Only one peritrichous ciliate with a pseudochitinous lorica was observed in the gills of bait shrimp. This ciliate was tentatively identified (Kudo, 1954) as a species of <u>Lagenophrys</u> (Fig. 5) having the peristomal margin connected with the inner margin of the lorica aperture and a stalked disk extending out of the lorica. The lorica also possessed a flattened adhering surface and a short neck with a convex surface.

The seasonal incidence of <u>Lagenophrys</u> sp. is presented in Figure 4. <u>Lagenophrys</u> sp. occurred in shrimp during all months except August and was most frequent in May and June. All of the shrimp examined during June showed this organism. Unlike other ciliates observed in association with shrimp, <u>Lagenophrys</u> sp. does cause tissue damage, evoking a strong cellular response from the host (Fig. 6). <u>Lagenophrys</u> sp. appears to digest the cuticle of the gills and often will become encysted in the underlying tissue. A strong host response is indicated by congestion of hemocytes in the affected gill filament and formation of copious amounts of melanin. The melanin imparts a grossly observable brown discoloration to the gills.

Two species of stalked, non-loricated peritrichous ciliates, <u>Epistylis</u> sp. (Fam. Epistylidae) and <u>Zoothamnium</u> sp. (Fam. Vorticellidae), were observed attached to the gills. <u>Epistylis</u> sp. is normally on dichotomous, non-contractile stalks, while in <u>Zoothamnium</u> sp. (Fig. 7) the myonomes of all stalks of a colony are continuous so that the entire colony contracts or expands simultaneously (Kudo, 1954).

<u>Epistylis</u> occurred in only four of the white shrimp examined (two in March and two in September), while <u>Zoothamnium</u> sp. (Table 3) occurred in every month with the highest incidence in May, June, and July 1974. No histological damage attributable to <u>Zoothamnium</u> sp. was demonstrated (Fig. 8); however, heavy infestation of the gill imparted a grossly observable brown discoloration.

3. Sporozoa

a. Gregarinida

The gregarine observed in the digestive tract was identified as <u>Nematopsis</u> <u>penaeus</u> Kruse (1959). The incidence of <u>N. penaeus</u> ranged from a low of 80% in August and September to 95 or 100% in all other months. The occurrence in all shrimp examined was 95.8% (Table 4).

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The sporozoite stages of <u>N. penaeus</u> were observed in the gastric mill and midgut (Fig. 9). The gametocyte stages were only found in the hindgut or hindgut gland (Fig. 10). No evidence of intracellular invasion nor of other histological damage was observed.

The abundance of <u>N</u>. <u>penaeus</u> in brown and white shrimp is presented in Table 5. The data indicate that the gregarine is much more abundant in the digestive tract of white shrimp during the months of December to March. Although incidence was comparable between the two shrimp species (Table 5), the gregarine was more abundant in the white shrimp than in the browns.

b. Microsporidia

During this study, only two shrimp exhibited microsporidosis. The first was a white shrimp examined in March 1974 that was determined to be infected with <u>Nosema nelsoni</u>. The second microsporozoan occurred in a brown shrimp examined in May 1974 and was identified as <u>Thelohania</u> sp. The spores of <u>Thelohania</u> sp. were not confined to the ovarian tissue but were widespread throughout the musculature. Both animals displayed the classic white musculature or "cotton" appearance characteristic of microsporidosis.

D. Helminths

1. Nematoda

The larval nematodes observed in shrimp were initially identified in shrimp from Texas coastal waters as <u>Contracecum habena</u> by Corkern (1970). Recently, however, Overstreet (1973) in closer taxonomic examinations of the nematodes places them in the genus <u>Thynascaris</u>. Because of the differences in opinion of the taxonomic classification, observations presented here will refer to the organisms simply as nematodes. Nematodes were found in 10 to 100% of all samples except in November (Table 6). The larvae were observed in all tissues or organs examined except the musculature, with the highest incidence occurring in the dorsal gland (Table 7). Seasonally, the percent occurrence was highest during January through March.

The nematodes were also more abundant in February and March, and most were located in the dorsal gland (Table 8). For instance, 460 of the 500 total larval nematodes were observed in the dorsal gland of the 20 bait shrimp examined during March. The nematodes were not in the lumen of this gland, but were embedded in the connective tissue; no inflammatory response to any of the nematodes was ever observed (Fig. 11). The nematodes observed in the gills were located in hemolymph vessels, while those observed in the hindgut, hindgut gland, midgut, and foregut were in the lumen of the digestive tract.

2. Cestoda

Numerous unidentified Tetraphyllidean procercoid larvae (Kruse, 1959) were observed in the midgut of bait shrimp (Fig. 12). The highest incidence occurred in August and September in which 31.2% of the shrimp examined were infected. The numbers of cestode larvae in those shrimp infected ranged from 12 to 306. Unlike the gregarines and nematodes observed in the digestive tract, the procercoid larvae apparently became attached to the gut mucosa (Fig. 13).

The life cycle of the trypanorhynchid cestode, <u>Prochristianella</u> <u>hispida</u>, has been discussed in detail (Aldrich, 1965; Overstreet, 1978) as has the host reaction to larval stages (Sparks and Fontaine, 1973). The incidence of <u>Prochristianella</u> larvae (Fig. 14) in white and brown shrimp is presented in Table 9. The incidence in all shrimp remained fairly consistent during this study; likewise, little differences occurred in incidence between the two species of shrimp. The highest incidence was 100% of all white shrimp during July and August. The numbers of cestode larvae ranged from 1 to 35 in white shrimp and 1 to 31 in brown shrimp. The majority of the larvae were encysted in the fibrous connective tissue sheath that surrounds the hepatopancreas; however, larval cysts were

observed in the hindgut, hindgut gland, and in the heart.

Several unidentified Cyclophylidean larvae (personal communication, William H. Wardel, Texas A&M University at Galveston) were observed encysted in the wall of the midgut of one shrimp. When dissected and placed on a glass slide, coverslip pressure was enough to evert the acetabulum (Fig. 15). These larvae were similar to those identified as Cyclophyllidea in brown and white shrimp by Corkern (1978).

3. Trematoda

A metacercaria identified as <u>Opecoeloides fimbriatus</u> by Kruse (1959) was observed in the hepathopancreas of one brown shrimp examined in June 1974. The trematode was non-encysted (Fig. 16) and appeared to move freely through the interstitial spaces in the hepatopancreas.

DISCUSSION

The observations on incidence and seasonal abundance of bacteria, commensals, and parasites of penaeid shrimp presented here should aid in future recognition and diagnosis of diseases encountered in natural shrimp populations. Many of the bacteria isolated from shrimp in this study have been shown to be pathogenic to penaeid shrimp (Lightner and Lewis, 1975); however, none of the shrimp from which these bacteria were isolated displayed the clinical signs of a bacterial septicemia as described by Lightner and Lewis (1975).

Additionally, many of the brown gill syndrome problems observed in shrimp during this study were not recognized by gross symptoms as are those published by Lightner et al. (1976). Often, no gross signs were observable in live bait shrimp, but closer examination of the gills showed them to be highly infested with the filamentous organism (Fig. 2). Possibly the brown gill syndrome may be a result of a synergistic complex of commensal and parasitic organisms, rather than one organism causing direct histopathological lesions.

Penaeid shrimp from naturally occurring populations are evidently subjected to a wide variety of parasites, potentially pathogenic bacteria,

and commensals that may cause respiratory problems. Apparently, most of the wild shrimp are able to cope with these situations and should be the subject of detailed investigations to determine what, if any, immune or other responses are responsible for their apparent resistance. Such knowledge should form a basis for sound shrimp pathology investigations.

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A <u></u>	Temperature	Salinity		Sex		Average
Date	(°C)	(ppt)	Male	Female	Total	(X, mm)
Nov. 1973	3 24	15	2	6	8	102.5
Dec.	20	15	8	12	20	107.8
Jan. 1974	18	17	1	9	10	95.3
Feb.	18	15	8	12	20	99.1
Mar.	20	14	11	9	20	105.2
Apr.	24	20	10	10	20	103.8
Мау	28	18	7	13	20	81.4
June	28	20	11	9	20	95.3
July	30	27	10	10	20	98.2
Aug.	29	28	5	15	20	118.4
Sept.	24	22	10	10	20	114.6
Oct.	24	22	6	14	20	102.6
Nov.	20	19	6	14	20	106.0
Sum	307	252	95	143	238	1,330.2
Mean	23.6	19.4	7.3	11.0	18.3	102.3

Table 1. Temperatures, salinities, sex and average total length of bait shrimp (white and brown shrimp combined).

Table 2. The antibiotic sensitivity of seven of the more common bacteria isolated from bait shrimp (brown and white shrimp combined). $\frac{a}{a}$

Isolate	A	С	сı	Na	Gm	PB	L	т	N	Dm	F/M	OL
Vibrio alginolyticus	<u></u> Ra∕	s	R	R	S	R	R	R	R	R	S	R
Vibrio anguillarum	R	S	R	R	S	R	R	R	R	R	S	R
Aeromonas formicans	R	s	R	R	R	R	R	R	R	R	S	R
Aeromonas shigelloides	s <u>b</u> /	s	S	s	S	s	R	R	R	R	S	R
Pseudomonas piscicida	R	s	S	R	S	S	R	R	R	R	R	S
<u>Pseudomonas</u> stutzeri	R	S	S	S	S	S	R	R	R	R	R	S
Lucibacterium harveyi	R	S	R	R	S	R	R	R	R	R	S	R

A - aureomycin; C - chloromycetin; Cl - colymycin; Na - nalidixix;

Gm - gentamycin; PB - polymycin; T - teramycin; N - neomycin;

Dm - demeclocycline; F/M - furadantin/ macrodantin; OL -oleandomycin

 $\underline{a}/R = resistant$

'S = sensitive

Table 3. Number of shrimp infected and incidence by month of Zoothamnium sp., Lagenophrys sp., suctorian, and an unidentified filamentous organism observed in gills of bait shrimp (white and brown shrimp combined).

	Zoothamnium		۲۹۵۳ ۱۵۵۵ (۱۳۵۵ ۲۰۰۵) ۲۹۵۵ (۲۹۵۵ ۲۹۵۵) ۲۹۵۵ (۲۹۵۵ ۲۹۵۵) ۲۹۵۵ (۲۹۵۵ ۲۹۵۵) ۱۳۹۵ (۲۹۵۹ ۲۹۵۵ ۲۹۵۹ ۲۹۵۵ ۲۹۵۹ ۲۹۵۹ ۲۹۵۹ ۲	Lagenc	phrys	Suctor	ian	Filamentous Organism		
	Nu	mber	Number	an na fallan di kanan da manan da manan da manan da manan da kanan da kanan da kanan da kanan da kanan da kanan	Number	anna an an Anna	Number		Number	
	of	Shrimp	of Shrimp	Percent	of Shrimp	Percent	of Shrimp	Percent	of Shrimp	Percent
Date	Ex	amined	Infected	Occurrence	Infected	Occurrence	Infected	Occurrence	Infected	Occurrence
			an na an a	an tanan kanan	andre and an	ann an tha ann an tha ann ann an tha ann an t	alar ya ana amin'ny fanina amin'ny fany any fan Alfred and an ana ana amin'ny fanina amin'ny fanina amin'ny fan	ĸĊĬĊŢġĸĸŊĊŢŢĸĸŢĸĬĸŶŎĸĊŎĬĬŔġġĸĸŊġĸĸŢŊŶĸĬĸĬĸĬĬĬĬĬĬĬĬĸŎĬĬĸĸġĸŢĸŢĬĬĬĬĬŎ	na na manga mangana na mangang kang kang na mangang na mangang kang kang kang kang kang kang kan	an a
Nov.	1973	8	6	75	5	62.5	4	50	0	0
Dec.		20	2	10	19	95	14	70	0	0
Jan.	1974	10	6	60	5	50	0	0	0	0
Feb.		20	13	65	8	40	0	0	0	0
Mar.		20	7	35	6	30	0	0	1	5
Apr.		20	8	40	4	20	0	0	0	0
May		20	16	80	17	85	2	10	1	5
June		20	20	100	20	100	9	45	3	15
July		20	17	85	5	25	2	10	8	40
Aug.		20	9	45	0	0	1	5	11	55
Sept	•	20	13	65	4	20	0	0	0	0
Oct.		20	15	75	12	60	9	45	1	5
Nov.	····	20	12	60	10	50	11	55	4	20
Tota	1	238	144	60.5	115	48.3	52	21.8	29	12.2

		No. of		No. ar	nd % o	f Shrim <u>r</u>	o Infect	ed		
		Shrimp	Foregut		Midg	ut	Hindgu	t	Total	
Da	te	Examined	No.	*	No.	*	No.		No.	8
Nov.	1973	8	5	62	7	88	6	75	8	100
Dec.		20	19	95	19	95	20	100	20	100
Jan.	1974	10	5	50	10	100	8	80	10	100
Feb.		20	13	65	20	100	19	95	20	100
Mar.		20	7	35	17	85	18	90	19	95
Apr.		20	1	5	19	95	14	70	20	100
May		20	3	15	20	100	18	90	20	100
June		20	4	20	11	55	16	80	19	95
July		20	3	15	19	95	17	85	20	100
Aug.		20	0	0	11	55	13	65	16	80
Sept	•	20	1	5	16	80	14	70	16	80
Oct.		20	1	5	18	90	17	85	20	100
Nov.		20		5	15	75	17	85	20	100
Tota]	L	238	63	26.5	202	84.9	197	82.8	228	95.8

Table 4. Incidence of the gregarine <u>Nematopsis</u> penaeus in the digestive tract of bait shrimp (white and brown shrimp combined).

Average Average Average Number Total Number of Number Total Number of Shrimp Number of N. penaeus of Shrimp Number of N. Date Examined N. penaeus Per Shrimp Examined N. penaeus Per Nov. 1973 8 4077 509.6 0 0	verage umber of <u>penaeus</u> er Shrimp
Number of ShrimpTotalNumber of Number ofNumber of N.Number of Per ShrimpNumber of N.Number of N.DateExaminedN.penaeusPer ShrimpExaminedN.penaeusNov. 197384077509.600	umber of <u>penaeus</u> er Shrimp
of Shrimp Number of <u>N. penaeus</u> of Shrimp Number of <u>N.</u> Date Examined <u>N. penaeus</u> <u>Per Shrimp Examined</u> <u>N. penaeus</u> <u>Pe</u>	<u>penaeus</u> er Shrimp
Date Examined N. penaeus Per Shrimp Examined N. penaeus Pe	er Shrimp
Nov. 1973 8 4077 509.6 0 0	0
	Ū
Dec. 20 8514 425.7 0 0	0
Jan. 1974 10 3890 389.0 0 0	0
Feb. 20 7335 366.8 0 0	0
Mar. 20 4906 245.3 0 0	0
Apr. 7 2051 293.0 12 ^a / 1515	126.2
May 1 505 505.0 19 6217	327.2
June 0 0 0 20 967	48.4
July 4 536 134.0 16 1612	100.8
Aug. 14 454 32.4 6 279	46.5
Sept. 18 1171 65.1 1 <u>b</u> / 205	205.0
Oct. 20 2798 139.9 0 0	0
Nov. 20 1722 86.1 0 0	0

Table 5.	Numbers of Nematopsis penaeus observed in the digestive tract	of
	bait shrimp (white shrimp and brown shrimp combined).	

 $\frac{a}{-}$ one pink shrimp, P. duorarum, was examined, not included. $\frac{b}{-}$ one seabob, Xiphopeneus kroyeri, was examined, not included.

		Number			Number c	ff Larval	Nematodes	Observ	ed	
		of Shrimp				······································	Hepato-	Dorsal		Percent
Dat	te	Examined	Gills	Hindgut	Midgut	Foregut	pancreas	Gland	Muscle	Infected
						······································				
Nov.	197:	38	0	0	0	0	0	0	0	0
Dec.		20	0	0	0	1	1	0	0	10
Jan.	1974	1 10	0	0	0	0	4	2	0	60
Feb.		20	0	0	2	0	0	15	0	85
Mar.		20	0	2	1	8	2	20	0	100
Apr.		20	0	2	0	5	3	11	0	55
May		20	0	0	0	1	2	1	0	15
June		20	0	0	1	0	0	0	0	5
July		20	0	0	0	0	4	10	0	50
Aug.		20	1	0	0	1	2	6	0	40
Sept.	•	20	0	0	0	0	1	5	6	30
Oct.		20	0	0	0	0	1	5	6	30
Nov.		20	0	0	0	1	1	9	0,	45
Total	•	238	1	4	4	17	21	84	12	41.2
Frequ Occur	iency renc	of e (%)	0.4	1.7	1.7	7.1	8.8	35.3	5.0	41.2

Table 6.	Occurrence of	f larval	nematodes	observed	in	bait	shrimp	(white	and
	brown shrimp	combined	d).						

Date	No. of Shrimp Examined	No. of Shrimp Infected	% Infected	Average No. Nematodes in Infected Shrimp
		White Shrimp		
		<u>mirce our rup</u>		
Nov. 1973	8	0	0	0
Dec.	20	2	10.0	1.0
Jan. 1974	10	6	60.0	4.3
Feb.	20	16	80.0	16.5
Mar.	20	20	100.0	25.0
Apr.	7	7	100.0	21.9
Мау	1	0	0	0
June				
July	4	2	50.0	1.5
Aug.	14	4	29.0	5.5
Sept.	18	6	33.3	1.7
Oct.	20	5	25.0	1.6
Nov.		10	50.0	3.5
Total	162	78	48.1	13.1
		Brown Shrimp		
Apr.	12	4	33.3	2.0
May	19	3	16.0	1.3
June	20	1	5.0	1.0
July	16	8	50.0	5.2
Aug.	6	4	67.0	4.0
Sept.	1	0	0	0
Total	74	20	27.0	3.2
			·	

Table	7.	The monthly incidence of larval nematodes in bait shrimp, Penaeus
		setiferus and Penaeus aztecus, with average number of worms per
		infected animal.

	No. of		No	of Ne	matodes O	bserved		
	Shrimp					Hepato-	Dorsal	•
Date	Examined	Gills	Hindgut	Midgut	Foregut	pancreas	Gland	Total
Nov. 197	38	0	0	0	0	0	0	0
Dec.	20	0	0	0	1	1	0	2
Jan. 197	4 10	0	0	0	0	10	15	25
Feb.	20	0	0	2	0	0	263	265
Mar.	20	0	2	1	35	2	460	500
Apr.	20	0	2	0	8	6	145	161
May	20	0	0	0	1	2	1	4
June	20	0	0	1	0	0	0	1
July	20	0	0	0	0	7	38	45
Aug.	20	1	0	0	1	2	26	30
Sept.	20	0	0	0	0	1	7	8
Oct.	20	0	0	0	0	1	7	8
Nov.	20	0	0	0	1	1	31	33
Total	238	1	4	4	47	33	993	1082
Frequence	ey of ce (%)	0.09	0.36	0.36	4.34	3.05	91.7	7

Table 8. Number of larval nematodes in bait shrimp (white and brown shrimp combined).

·	Pe	naeus setiferus		Penaeus aztecus			
Date	Infected	No. Examined	8	Infected	No. Examined	ક	
Nov. 1973	5	8	62.5	0	0	0	
Dec.	12	20	60.0	0	0	0	
Jan. 1974	4	10	40.0	0	0	0	
Feb.	15	20	75.0	0	0	0	
Mar.	10	20	50.0	0	0	Ö	
Apr.	3	7	42.8	1	12	8.3	
May	0	1	0	8	19	42.1	
June	0	0	0	10	20	500	
July	4	4	100.0	10	16	62.5	
Aug.	14	14	100.0	5	6	83.3	
Sept.	13	18	72.2	0	1	0	
Oct.	8	20	40.0	0	0	0	
Nov.	6	20	30.0	0	0	0	
Total	94	162	58.0	34	74	45.9	

Table 9. Occurrence of the larval trypanorhynchid cestode, Prochristianella penaei, in bait shrimp, Penaeus setiferus and Penaeus aztecus.

FIGURES

- 1. The incidence by months of <u>Vibrio</u>, <u>Pseudomonas</u>, and <u>Aeromonas</u> isolated from the hemolymph of bait shrimp.
- 2. Unidentified filamentous organism attached to the endite of basipodite of the first maxilla. Wet mount, no stain, X50.
- 3. Unidentified suctorian attached to tip of gill filament (F). Wet mount, no stain, X75.
- 4. The incidence of <u>Zoothamnium</u>, <u>Lagenophrys</u>, suctorian, and the filamentous organism observed attached to the gill of bait shrimp.
- 5. <u>Lagenophrys</u> sp. (L) encysted in gill filament. Note intense melanization of the filament by hemocytes. Wet mount, no stain, X50.
- Histological section showing <u>Lagenophrys</u> (L) digesting into a gill filament (F). H&E, X50.
- 7. Heavy infestation of <u>Zoothamnium</u> on the gills. Wet mount, no stain, X50.
- Histological preparation of <u>Zoothamnium</u> (Z) attached to the gills (G).
 Note no inflammatory response as with Lagenophrys. H&E, X75.
- 9. <u>Nematopsis penaeus</u> sporozoite in midgut of shrimp. Wet mount, no stain, X100.
- 10. <u>Nematopsis penaeus</u> gametocyte in hindgut of shrimp. Wet mount, no stain, X50.
- 11. Histological preparation of dorsal gland showing sections of larval nematodes (N). Note the larvae are in the connective tissue of the gland and not in the lumen (L). H&E, X125.

- 12. Unidentified cestode procecoid in midgut of shrimp. Wet mount, no stain, X200.
- 13. Histological preparation of midgut showing unidentified cestode attached to gut lining. H&E, X125.
- 14. Trypanorhynchid larvae, <u>Prochristianella hispida</u>, teased from a capsule on the hepatopancreas. Wet mount, no stain, X450.
- 15. Cyclophilidean larvae encysted in the wall of the midgut. Wet mount, no stain, X75.
- 16. Trematode metacercaria from hepatopancreas. Wet mount, no stain, X125.

1. The incidence by months of <u>Vibrio</u>, <u>Pseudomonas</u>, and <u>Aeromonas</u> isolated from the hemolymph of bait shrimp.



1. The incidence by months of <u>Vibrio</u>, <u>Pseudomonas</u>, and <u>Aeromonas</u> isolated from the hemolymph of bait shrimp.

PERCENT INCIDENCE

2. Unidentified filamentous organism attached to the endite of basipodite of the first maxilla. Wet mount, no stain, X50.



 Unidentified suctorian attached to tip of gill filament (F). Wet mount, no stain, X75.



4. The incidence of <u>Zoothamnium</u>, <u>Lagenophrys</u>, suctorian, and the filamentous organism observed attached to the gill of bait shrimp.



4. The incidence of <u>Zoothamnium</u>, <u>Lagenophrys</u>, suctorian, and the filamentous organism observed attached to the gill of bait shrimp.

5. <u>Lagenophrys</u> sp. (L) encysted in gill filament. Note intense melanization of the filament by hemocytes. Wet mount, no stain, X50.



 Histological section showing <u>Lagenophrys</u> (L) digesting into a gill filament (F). H&E, X50.



 Heavy infestation of <u>Zoothamnium</u> on the gills. Wet mount, no stain, X50.



 Histological preparation of <u>Zoothamnium</u> (Z) attached to the gills (G). Note no inflammatory response as with Lagenophrys. H&E, X75.

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9. <u>Nematopsis penaeus</u> sporozoite in midgut of shrimp. Wet mount, no stain, X100.



10. <u>Nematopsis penaeus</u> gametocyte in hindgut of shrimp. Wet mount, no stain, X50.



11. Histological preparation of dorsal gland showing sections of larval nematodes (N). Note the larvae are in the connective tissue of the gland and not in the lumen (L). H&E, X125.



12. Unidentified cestode procecoid in midgut of shrimp. Wet mount, no stain, X200.

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 Histological preparation of midgut showing unidentified cestode attached to gut lining. H&E, X125.



14. Trypanorhynchid larvae, <u>Prochristianella hispida</u>, teased from a capsule on the hepatopancreas. Wet mount, no stain, X450.

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15. Cyclophilidean larvae encysted in the wall of the midgut. Wet mount, no stain, X75.

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16. Trematode metacercaria from hepatopancreas. Wet mount, no stain, X125.

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