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# A Septicemic Bacterial Disease Syndrome of Penaeid Shrimp

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*ABSTRACT—A disease syndrome of penaeid shrimp characterized by the presence of a septicemic phase is described. Mortality due to the disease in infected shrimp populations typically ranged from 10 to 50 percent, but occasionally reached 100 percent.* Vibrio alginolyticus, V. anguillarum, *and an* Aeromonas *sp. were isolated from moribund hatchery-reared white shrimp (*Penaeus setiferus*) and brown shrimp (*P. aztecus*) and from wild white, brown, and pink shrimp (*P. duorarum*). Each of these organisms was found to be pathogenic to shrimp when administered by intramuscular injection.*

## INTRODUCTION

During 1972 and 1973, several large mortalities occurred in hatchery-reared brown shrimp (*Penaeus aztecus*) and white shrimp (*P. setiferus*) apparently as the result of a disease. A similar, if not identical disease, was observed in wild brown, white, and pink shrimp (*P. duorarum*) obtained from commercial bait dealers in Galveston. Mortalities from this disease ranged from a few shrimp lost per day in some cases to nearly 100 percent in other "die-offs." In these epizootics *Vibrio alginolyticus* was the organism most commonly isolated from diseased shrimp.

Vibrionic infections have been implicated as a major cause of mortality in juvenile penaeids in shrimp culture (Sindermann, 1971). *Vibrio parahaemolyticus*, the cause of an infectious food poisoning syndrome in Japan (Nickelson and Vanderzant, 1971) was isolated from white shrimp taken from Galveston Bay, Tex. (Vanderzant, Nickelson, and Parker, 1970). That same organism was found to be pathogenic to brown shrimp when bits of frozen white shrimp containing the organism were fed to brown shrimp, or when cultures of the organism were added to aquaria with brown shrimp.

*V. parahaemolyticus* has been isolated from diseased blue crabs (*Callinectes sapidus*) in Chesapeake Bay (Krantz et al., 1969; Colwell et al., 1972). Furthermore, *V. parahaemolyticus* or a very similar organism was isolated from Gulf of Mexico and South Atlantic coastal water and sediment samples (Ward, 1968), indicating that this organism which is potentially pathogenic to shrimp and other crustaceans is ubiquitous in many estuaries.

Lewis (In press) reported experiments in which adult brown shrimp were challenged with a field isolate of *V. anguillarum*. One-tenth ml of a 24-h broth culture diluted one hundredfold and introducted by injection beneath the dorsal carapace at the terminus of the rostral groove caused death of the shrimp within 5 days.

The microbial flora of brown and white shrimp from the Gulf of Mexico and from pond-reared brown shrimp have been studied (Vanderzant, Mroz, and Nickelson, 1970; Vanderzant et al., 1971). These studies indicated that the microbial flora of the Gulf shrimp and pond shrimp differ slightly. Bacterial counts of pond shrimp were reported to be much lower than those from Gulf shrimp (Vanderzant, Mroz, and Nickelson, 1970). The coryneforms (species of *Corynebacterium*, *Arthrobacter*, and *Microbacterium*) and to a lesser extent *Vibrio* were the predominant isolates from fresh pond-reared brown shrimp. In contrast, the microbial flora of Gulf shrimp was dominated by coryneforms and species of *Pseudomonas*, *Moraxella*, and *Micrococcus*.

This paper presents studies in which two *Vibrio* species were isolated from and found to be pathogenic to the penaeid shrimp, *Penaeus aztecus*, *P. setiferus*, and *P. duorarum*. Evidence is also presented that an *Aeromonas* sp. may cause a similar disease syndrome in these animals.

## METHODS OF ISOLATION AND CULTURE

Bacteria were isolated from diseased laboratory-reared brown shrimp (*P. aztecus*) and white shrimp (*P. setiferus*) and from wild brown, white, and pink shrimp (*P. duorarum*) obtained by trawling from the Gulf or from local bait camps on West Galveston Bay. Juvenile and adult stages were represented. Cultures were obtained by extraction of hemolymphs by cardiac

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puncture in moribund shrimp over 40 mm in total length from the tip of rostrum to the tip of telson. In shrimp under 40 mm in total length, cultures were obtained by tissue impression of small pieces of abdominal muscle tissue onto the isolation medium. Shrimp that had been dead 1-2 h before necropsy were not considered suitable for bacterial isolation because of the advanced state of autolysis of the hepatopancreas and heart, and contamination of the hemolymph by enteric microorganisms.

The isolation media were tryptic soy agar (Difco Laboratories[1]) with 2 percent sodium chloride and tryptic soy agar with 7 percent NaCl. The inoculated media were incubated for 24-48 h at 28°C. Bacterial colonies were then transferred to fresh tryptic soy agar with 2 percent NaCl and streaked for individual colonies and incubated as described previously. This process was repeated until pure cultures were obtained of each organism present in the initial isolate.

All the bacteria studied were Kovac's oxidase positive, were motile by polar flagella, and initially required the presence of at least 2 percent NaCl in the medium for growth. None of the fermentative bacteria produced gas in glucose, lactose, sucrose, or mannitol. Those organisms which failed to produce lysine decarboxylase were beta hemolytic on 5 percent bovine blood agar and on the basis of their ability to produce arginine dihydrollase, 2-3 butanediol, gelatinase, and indole were identified as *Aeromonas* sp. (Eddy, 1960; Eddy and Carpenter, 1964; Schubert, 1967). Those organisms identified as *Vibrio* sp. were sensitive to 2, 4-diamino-6, 7- diisopropyl pteridine phosphate (Schubert, 1962), produced lysine and ornithine decarboxylase, indole, fermented sucrose, and grew in trypticase soy broth containing 10 percent NaCl. Further identification of the isolates was accomplished using methods described elsewhere (Lewis, 1973).

## INFECTIVITY EXPERIMENTS

Heavily streaked 24- to 48-h cultures of each bacterial isolate were harvested in sterile saline (2 percent NaCl) and diluted to a standard concentration equal to a particulate suspension that allows 70 percent light transmission at a wavelength of 520-540 nm (Perkin-Elmer spectrophotometer model 124-Coleman). This standard suspension of bacteria contained approximately $10^7$ bacterial cells/ml and was either administered directly to experimental shrimp or diluted by tenfold serial dilutions prior to being administered to shrimp.

Shrimp used in infectivity experiments were brown, white, and pink shrimp juveniles averaging 95 mm total length. The shrimp were obtained from laboratory-reared stocks or from local bait camps. Addition of bacterial isolates to the food given to the shrimp proved to be an unsuccessful means of infection. Therefore, inoculation by intramuscular injection was adopted as the most reliable method of ascertaining the pathogenicity of bacterial isolates.

Experimental shrimp were inoculated intramuscularly between the fifth and sixth abdominal segments with 0.05 ml of a bacterial suspension (approximately $10^5$ bacterial cells) using a 1-cc tuberculin syringe. Control shrimp were given 0.05 ml sterile saline (2 percent NaCl) in the same location.

Experimental and control shrimp were maintained in 60-liter glass aquaria at 25-30°C for up to one week. Shrimp were checked twice daily for clinical signs of disease and mortality. Dead shrimp were removed.

## CLINICAL SIGNS OF BACTERIAL DISEASE

The first apparent clinical sign of a lethal bacteremia was a gradual change from the usual colorless, translucent appearance of the musculature, particularly of the abdominal musculature, to a whitish-opaque coloration. Some animals examined also showed melanization of gill filaments, cuticular lesions, and ventrolateral edges of the carapace. A slight darkening of the dorsal portions of the integument (due to expansion of integumental melanophores) and a reddening of the pereiopods and the pleopods (due to expansion of integumental erythrophores) was usually apparent in moribund or freshly dead shrimp. Moribund shrimp commonly exhibited a pronounced dorsal flexure of the abdomen with the second and third abdominal segments at the apex of the flexure.

Behavioral signs of stress associated with the disease became more apparent as the disease progressed. These behavioral signs included reduced swimming activity, disorientation while swimming, and swimming on one side. Eventually, affected shrimp came to rest motionless on the bottom, some in an upright position supported by the pereiopods, pleopods, and telson, while others lay on their side. Some of these shrimp could be induced to brief periods of swimming activity by prodding. Death occurred usually 2-4 h after the shrimp had become lethargic. Occasionally shrimp remained in the upright position even after death.

Hemolymph drawn with a 1-cc tuberculin syringe directly from the heart of moribund shrimp typically would not clot as rapidly as hemolymph drawn from control shrimp. Hemolymph drawn in this manner from healthy shrimp clots very rapidly, often before it can be expelled from the syringe. The clotting of hemolymph from bacteremic shrimp is slower, requiring from 5 to 10 min after being drawn; hemolymph from some infected shrimp had not clotted 1 h after being drawn. Hemolymph drawn from most moribund shrimp having a bacteremia was slightly turbid in appearance and lacked the blue coloration that appears in clotted hemolymph of healthy shrimp. Giemsa stained hemolymph smears from moribund bacteremic shrimp contained hemocytes in greatly reduced numbers compared to normal shrimp. Gram stained hemolymph smears from the same animals showed the presence of numerous gram negative rods.

## BACTERIA ISOLATED

Pure cultures of bacteria were usually obtained when hemolymph was drawn directly from the heart of moribund shrimp (Table 1). Cultures made from impression smears of small pieces of muscle tissue aseptically removed from the abdomen of small shrimp (under 40 mm total length) also frequently provided pure cultures of the presumed causative agent.

*Vibrio alginolyticus* and *V. anguillarum* were the most prevalent organisms isolated from shrimp with clinical signs of a bacteremia taken from

[1]Use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

**Table 1.—Organisms isolated and source of penaeid shrimp exhibiting clinical signs of a bacterial septicemia.**

| Date | Isolate number | Organism(s) isolated | Species[1] | Source, number of shrimp involved, and percent mortality |
|---|---|---|---|---|
| 8/14/72 | 30 | *Vibrio alginolyticus* | B (40 mm) | Lab-reared; 150; 20% |
| 9/21/72 | 31 | *V. alginolyticus*, *V. anguillarum* | W (100 mm) | Local bait camp; 50; 50% |
| 1/9/73 | 53 | *V. alginolyticus* | B (26 mm) | Lab-reared; 100,000; 99% |
| 3/8/73 | 58 | *V. alginolyticus* | B (58 mm) | Lab-reared; 200; 40% |
| 3/16/73 | 59 | *V. anguillarum*, *Aeromonas* sp. | B (90 mm) | Lab-reared; 10; 10% |
| 3/18/73 | 61 | *V. alginolyticus* | W,P,B (100 mm) | Local bait camp; 60; 50% |
| 3/19/73 | 63 | *V. alginolyticus*, *Aeromonas* sp. | B (90 mm) | Lab-reared; 10; 30% |
| 3/20/73 | 64 | *V. alginolyticus* | B (41 mm) | Lab-reared; 1,500; 99% |
| 3/28/73 | 67 | *V. alginolyticus* | B (120 mm) | Gulf of Mexico[2]; 7; 100% |
| 4/18/73 | 73-74 | *Vibrio* sp. | W,P (120 mm) | Local bait camp; 100; 10% |

[1]B=brown shrimp (*Penaeus aztecus*). W = white shrimp (*P. setiferus*), and P = pink shrimp (*P. duorarum*). Average total length in parentheses.
[2]Held in laboratory tanks 40 days prior to onset of clinical disease.

Galveston Bay and vicinity and from hatchery-reared shrimp showing the same disease syndrome (Table 1). *Vibrio parahaemolyticus* was not isolated. An *Aeromonas* sp. was isolated along with *V. anguillarum* and *V. alginolyticus* from hatchery-reared shrimp from one location, but were not present in isolates taken from shrimp from other locations.

## PATHOGENICITY EXPERIMENTS

Twenty-four to 48-h cultures of *V. alginolyticus*, *V. anguillarum*, and an *Aeromonas* sp. were found to be pathogenic to juvenile white, brown, and pink shrimp when the inoculum was administered directly to the shrimp by intramuscular injection in the fifth abdominal segment. The standard inoculum used to test the pathogenicity of bacterial isolates to healthy penaeid shrimp usually caused the death of exposed shrimp within 24-48 h. Most strains of *V. alginolyticus* caused the death of all shrimp tested within 24 h of inoculation (Table 2).

Injections of tenfold serial dilutions of the standard bacterial suspension resulted in progressively delayed times of appearance of clinical signs of disease and mortality (Table 2). Dilutions greater than 1:100 failed to produce clinical signs of disease or mortality in an experiment using a field isolate of *V. alginolyticus*.

## DISCUSSION OF RESULTS

Every bacterial isolate obtained from obviously diseased penaeid shrimp was found to be pathogenic to healthy shrimp when administered directly by intramuscular injection. The virulence of the isolates varied, with some isolates causing 100 percent mortality in inoculated shrimp within 12 h while other isolates required considerably more time to kill the shrimp. Feeding of bacterial isolates to experimental shrimp seldom resulted in clinical disease. Other investigators have had similar difficulties in infectivity experiments. Lewis (In press) selected injection of *V. anguillarum* into experimental shrimp over other methods of exposure. Snieszko and Taylor (1947) were unable to infect American lobsters with *Gaffkya homari* introduced with the food, but succeeded in transmitting gaffkemia disease to healthy lobsters by injection of bacteria. It was later found that gaffkemia is transmitted only through ruptures in the integument and not through the consumption of infected food (Stewart and Rabin, 1970). This may also be the case in penaeid shrimp.

The presence of *Vibrio* species as part of the normal flora of pond-reared shrimp (Vanderzant et al., 1971) and the presence of these organisms in the water and sediments of estuaries (Ward, 1968) would tend to indicate that penaeid shrimp are resistant to infection by *Vibrio* by the oral route. Our experience has shown that handling of otherwise healthy hatchery-reared shrimp occasionally results in the onset of disease due, in most cases, to a *Vibrio* sp. The capture and holding in tanks of wild penaeid shrimp often result in occurrence of the same disease syndrome. Slight injuries resulting in interruption of the cuticle certainly occur when shrimp are subjected to rough handling or crowding in tanks. Cuticular injuries may provide a route of entrance for potentially pathogenic bacteria which are apparently a normal part of the microbial flora of shrimp (Vanderzant, Nickelson, and Parker, 1970; Vanderzant et al., 1971). Infection during ecdysis is also a distinct possibility.

*Vibrio parahaemolyticus* until now has been the most common *Vibrio* isolated from diseased crustacea. That organism is similar to *V. alginolyticus* but the latter differs from *V. parahaemolyticus* in its ability to swarm over the surface of the agar culture media within 12-24 h after inoculation at 28°C and in its ability to grow in media which does not contain salt.

The general gross appearance exhibited by moribund shrimp having a septicemic vibriosis has also been observed in moribund shrimp dying from causes other than a bacteremia. Gross clinical signs common to a bacteremia and to one or more other disease conditions include lethargy, areas of white discoloration of the abdominal musculature, a dorsal flexure of the abdomen, and redness of the pleopods and pereiopods. Shrimp exhibiting these signs and not having a bacteremia have been found to be suffering from hypoxia or anoxia due to the presence on the gills of large numbers of fouling organisms, the most

**Table 2.—Mortality data from pathogenicity experiments in which brown, white, and pink shrimp were exposed to bacterial isolates obtained from diseased shrimp.**

| Species[1] | Mean length (mm) | Number tested | Organism | Accumulated mortality at hours post-inoculation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 2 | 8 | 24 | 48 |
| W | 95 | 6 | C-53, *Vibrio alginolyticus* | 0 | | | 6 | |
| B | 150 | 1 | C-53, *V. alginolyticus* | 0 | | | 1 | |
| W | 95 | 6 | Saline control | 0 | | | 0 | |
| W,P | 96 | 14 | C-58, *V. alginolyticus* | 0 | 1 | 10 | 14 | |
| W,P | 95 | 5 | C-53, *V. alginolyticus* | 0 | 1 | 1 | 5 | |
| W,B | 92 | 5 | C-58, *V. alginolyticus* | 0 | 0 | 0 | 5 | |
| W,P | 84 | 5 | C-59, *V. anguillarum* | 0 | 0 | 0 | 4 | 5 |
| W | 95 | 5 | C-63A, *V. alginolyticus* | 0 | 0 | 4 | 5 | |
| W | 84 | 5 | C-63B, *Aeromonas* sp. | 0 | 0 | 3 | 5 | |
| W,B | 91 | 5 | C-64A, *V. alginolyticus* | 0 | 1 | 3 | 5 | |
| W | 81 | 5 | C-64B, *V. alginolyticus* | 0 | 0 | 3 | 5 | |
| W,P | 92 | 5 | Saline control | 0 | 0 | 0 | 0 | 0 |
| W,P,B | 95 | 5 | C-53, *V. alginolyticus* | 0 | | 1 | 5 | |
| W,P,B | 95 | 5 | 1/10, C-53, *V. alginolyticus* | 0 | 0 | 2 | 4 | |
| W,P,B | 95 | 5 | 1/100, C-53, *V. alginolyticus* | 0 | | 1 | 1 | 1 |
| W,P,B | 95 | 5 | 1/1,000, C-53, *V. alginolyticus* | 0 | | 0 | 0 | 0 |

[1]B = brown shrimp (*Penaeus aztecus*), W = white shrimp (*P. setiferus*), and P = pink shrimp (*P. duorarum*).

common of which is a stalked peritrich ciliate belonging to the genus *Zoothamnium*. Rigdon and Baxter (1970) described a similar condition in brown shrimp (*P. aztecus*) apparently due to anoxia with high temperature and handling stress as additive factors. These shrimp exhibited white discoloration and necrosis of the abdominal musculature. However, shrimp with a bacteremia can be distinguished from those suffering from anoxia or related conditions by the consistent presence of bacteria in the hemolymph.

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