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BACTERIA IN DISEASED DOVER SOLE

Previous histological examination of Dover sole (*Microstomus pacifious*) with fin erosion had suggested that the disease was not microbial in origin. However, some diseases that produce fin erosion in marine fish have been reported to be caused by species of *Vibrio* and *Pseudomonas* bacteria. The objectives of this study were to examine relationships between the presence of these and other known marine fish pathogens and the occurrence of fin erosion in Dover sole and to verify histological findings.

Dover sole with fin erosion were collected by trawl from the Palos Verdes shelf. Control Dover sole were collected from off Santa Catalina Island and Dana Point. Three samplings were conducted for each category of fish, and six specimens were examined during each cruise. Samples were taken from the fin, liver, kidney, and intestine. The fin was cleansed of slime with a sterile cotton-tip swab before fin samples were taken, and the surface of the fish was disinfected with 95 percent alcohol before internal samples were taken. Samples were inoculated directly onto blood agar plates with Zobell 2216 agar base and 10 percent sheep red blood cells, and quantitative measurements were made by dilution and plating from a known weight of minced tissue. The scheme used to identify or characterize the bacteria was essentially that of Shewan. This scheme is primarily designed to separate out the Gram-negative, aerobic mesophilic bacteria (i.e., bacteria requiring oxygen and a moderate temperature). We used the scheme of Sakazaki to help in the identification of *Vibrio* species. The information presented here is primarily from the last sampling conducted in March 1975.

Bacteria were essentially absent in samples taken from the liver and kidney of both healthy and diseased fish. However, bacterial counts were higher in the fin and intestine samples from the Palos Verdes specimens than in those from the controls (Table 1). We also noted a difference in the color of the fecal material of fish from Palos Verdes and Dana Point that may be a result of a difference in the diet of individuals from the two areas.

In the bacterial identification scheme. *Vibrio* and Pseudomonas species are oxidase-positive organisms. As species identification of many of the bacteria isolated was not possible, we looked at the numbers of oxidase-positive organisms in both diseased and healthy fish. Greater num-bers of oxidase-positive bacteria were found on the fins of Palos Verdes fish, but the percentage of these bacteria was greater in Dana Point fish (Table 2).

The ability of bacteria to break down red blood cells (hemolysis) is an indication of pathogenicity. We there-fore grouped the *Vibrio* species according to their hemolytic properties. Both the number and percent of hemolytic *vibrios* in fin samples were greater on the diseased fish; for samples of intestinal contents, the percent but not the number of hemolytic vibrios was greater in the diseased fish (Table 2).

Isolates that closely resemble known fish pathogens were found on both diseased and healthy fish. From Palos Verdes fish, we found species resembling both *Vibrio parahaemolytious* and *V. anguillarum*; from Dana Point fish, we found species resembling *V. anguillarum* and *V. alginolyticus*. Although nine isolates from the Palos Verdes fish were Gram-positive cocci-

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appearing in clusters, we concluded they were not *Staphylococcus aureus* because they were not hemolytic, did not give characteristic growth on Vogel-Johnson Agar, and did not exhibit coagulase activity.

Hydrogen sulfide producing bacteria, determined by TSI (triple sugar iron) reaction, were more prevalent in samples from Palos Verdes fish than in samples from Dana Point fish (no hydrogen sulfide producing bacteria were found in intestine samples from the Dana Point fish; Table 2). The numbers of these bacteria were very low in samples from Catalina fish.

If the fin erosion disease in the Dover sole were the result of a direct bacterial invasion, we would expect to find a particular bacterium in abundance at the site of the lesion. This was not the case. In fact, the bacterial population on the fins of Palos Verdes Dover sole was more diverse than that of Dana Point Dover sole. In addition, the absence of bacteria in both the kidney and liver samples indicates that the disease does not involve a systemic infection. Finally, the isolation of known fish pathogens does not mean that the disease is bacterial in origin because pathogens were found on both diseased and healthy fish.

Differences in numbers of total bacteria and of oxidase-positive bacteria, hemolytic *vibrios*, and hydrogen sulfide producing bacteria may be regional differences rather than differences associated with the condition of the fish. The increased number of total bacteria and the diversity of these bacteria on fish from Palos Verdes could be related to the enriched organic environment in which these fish live or to the fact that diseased fish appear to pro-duce less protective slime than healthy fish. The occurrence of hydrogen sulfide producing bacteria on the fins and in the intestine of Palos Verdes Dover sole suggests that these fish are living in an environment low in dissolved oxygen.

The next step will be to compare diseased and healthy Dover sole from the same regions and to explore the effects of hydrogen sulfide on the fins of the fish. Additionally, hydrogen sulfide producing bacteria and anaerobic bacteria from the sediments and from diseased and healthy fish should be examined. Finally, the pathogenicity of the *Vibrio* isolates from the healthy fish should be tested.

This study was conducted in conjunction with Eugene T. Manfredi, California State University at Long Beach, and data presented here will appear in his Master's thesis.

Table 1. Aerobic mesophilic marine bacteria associated with Dover sole with fin erosion from the Palos Verdes shelf and healthy Dover sole from off Dana Point

	Palos Verdes 19 Mar 75		Dana Point 31 Mar 75	
Sample No.	Description	Bacteria/Gram Of Tissue	Description*	Bacteria/Gram Of Tissue
Fin				
1	Severe erosion	3,330	WNL	380
2	Severe erosion	2,790	WNL	500
3	Moderate erosion	1,440	WNL	200
4	Moderate erosion	2,790	WNL	660
5	Severe erosion	1,890	WNL	220
6	Moderate erosion	1,980	WNL	320
Average		2,370		380
Intestine				
1	Empty	690	Full, brown material	71,000
2	Full, brown material	5,980	Full, brown material	28,600
3	Full, green material	92,000	Full, brown material	33,600
4	Full, green material	77,280	Full, brown material	29,400
5	Full, green material	10,120	Full, brown material	27,800
6	Full, green material	111,090	Full, brown material	39,000
Average		49,527		38,233
Kidney (Average)		<10		<10
Liver (Average)		<10		<10

WNL = within normal limits

Table 2. Oxidase-positive bacteria, hemolytic vibrios, and hydrogen sulfide producing bacteria associated with Dover-sole.

	Palos Verdes		Dana Point (Control)	
Oxidase-Positive Bacteria	Fin	Intestine	Fin	Intestine
Total oxidase-positive bacteria per gram of tissue	6,480	131,330	1,920	183,680
Total bacteria per gram of tissue	14,220	297,160	2,280	229,400
Percent oxidase-positive bacteria per gram of tissue	45.6	44.2	84.2	80.1
Hemolytic Vibrios				
Total vibrios per gram of tissue	1,980	3,450	7,600	123,500
Total hemolytic vibrios per gram of tissue	360	3,450	80	104,100
Percent hemolytic vibrios per gram of tissue	18.2	100	1.1	84.3
Hydrogen Sulfide Producing Bacteria				
Total H_2S-producing bacteria per gram of tissue	900	40,360	100	—*
Total bacteria per gram of tissue	14,220	297,160	2,280	
Percent H_2S-producing bacteria per gram of tissue	6.3	13.6	4.4	

*We examined intestine samples from Dana Point fish but found no H_2S-producing bacteria.