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QUANTITATIVE AND QUALITATIVE STUDIES OF GUT FLORA IN STRIPED BASS FROM ESTUARINE AND COASTAL MARINE ENVIRONMENTS

Richard D. MacFarlane,[1] John J. McLaughlin,[1] and G. L. Bullock[2]

ABSTRACT: Examination of the intestinal contents of 130 striped bass (*Morone saxatilis*) collected from the Hudson River and Long Island Sound during May to October 1981 showed that opportunistic fish pathogens—especially *Aeromonas hydrophila*—predominated in samples from both locations. Other isolates from both groups of striped bass included *Vibrio*, pseudomonads, flavobacteria, *Alcaligenes*, and enterics. Small numbers of *Micrococcus*, *Bacillus*, *Corynebacterium*, and *Acinetobacter* were also isolated. Total numbers of bacteria in the intestines were 100 to 1,000 times higher in striped bass from the Hudson River than in those from Long Island Sound.

INTRODUCTION

Bacterial diseases contribute to natural mortality and can be significant factors in population dynamics of fishes (Sindermann, 1970; Paperna and Zwerner, 1976). According to Cooper and Polgar (1981), disease sometimes adversely affects year-class success of striped bass (*Morone saxatilis*). Trust and Sparrow (1974) indicated that various species of gut microflora affect nutrition, growth, and susceptibility to disease among fishes. Although the flesh and body fluids of newly caught healthy fish are generally considered sterile, the gills and intestine usually harbor significant populations of bacteria. Moreover, the gut flora varies seasonally, both quantitatively and qualitatively. Several species of bacteria are opportunistic pathogens, and produce disease when fish are stressed (Shewan, 1961; Wedemeyer, 1974; Williams et al., 1974; Sindermann, 1978).

The present study was undertaken to gain quantitative and qualitative data on the bacterial flora of the gut of estuarine and coastal marine striped bass and to determine the percentage of opportunistic pathogens in gut flora.

MATERIALS AND METHODS

Representative striped bass were sampled from the lower Hudson River estuary and from Orient Point Harbor, Long Island Sound, New York, from May to October 1981. From nine to 12 fish were collected at each site each month.

Hudson River estuarine fish were all young of the year (~115 g and ~20 cm). Live fish were netted from the intake screens of two power plants—Indian Point II and III.

Fish from Long Island Sound were 2 yr old and averaged 352 g and 33 cm. Samples were obtained from stationary pound nets set 180 to 360 meters offshore in water of 25–28-ppt salinity.

Specimens were killed with tricaine methanesulfonate, topically disinfected with 70% isopropanol, and opened aseptically; the central portion of the intestine was then excised for culturing.

Sections of the intestine were individually homogenized and 10-fold dilutions were prepared in cold sterile 0.5% peptone solution. We pipetted 0.1-ml aliquots onto the surface of replicate brain heart infusion agar (BHIA) plates for culture of aerobic and facultative bacteria and onto Rimler-Shotts agar for isolation of *Aeromonas hydrophila* (Shotts and Rimler, 1973). Streak plates on BHIA were prepared also from kidneys and the liver. The BHIA spread plate cultures were incubated aerobically at 20 C, and the Rimler-Shotts plates at 37 C. Culture plates showing between 30 and 300 colonies were counted after 48 to 72 hr, and Rimler-Shotts plates were counted between 18 and 24 hr. We held all plates for 5 days to ensure detection of slow-growing organisms.

Colonies representing the most commonly

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TABLE 1. Distribution of gut flora in 130 striped bass from estuarine (Hudson River) and marine (Long Island) environments (May–October 1981).

Genus or group	Mean percentage distribution	
	Estuarine	Marine
Aeromonas	33	21
Pseudomonas	23	11
Vibrio	10	27
Enterobacters	15	14
Alcaligenes	9	5
Flavobacterium	5	18
Micrococcus	1	3
Corynebacterium	2	1
Bacillus	0.5	0
Acinetobacter	1	1

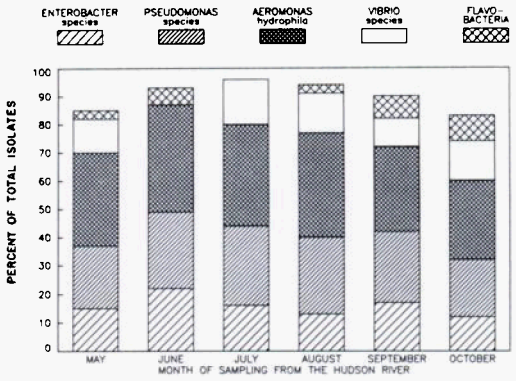

FIGURE 1. Monthly variation in the five most abundant intestinal bacteria in striped bass (*Morone saxatilis*) from an estuarine environment. (Cultures of *Vibrio* in June were lost before identification was completed.)

occurring morphological types were picked for identification, and less frequently occurring colony types were also picked to enable us to estimate variation. All colonies were restreaked to ensure purity.

Characterization of isolates was carried out in two stages. The organisms were first grouped according to the classification schemes of Shotts and Bullock (1975, 1976), or MacFaddin (1980). Additional characterization and identification was carried out with the Minitek Numerical Identification System (Baltimore Biological Laboratories, Cockeysville, Maryland 21030, USA).

RESULTS

Gram-negative rods made up 95% of the gut flora in fish from both environments, and about two-thirds of the organisms were species of the facultative fish pathogens *Aeromonas hydrophila*, *Pseudomonas*, and *Vibrio*. Aeromonads predominated in the estuarine environment but, as would be expected, *Vibrio* was the most abundant type in the marine environment (Table 1; Figs. 1, 2). The predominance of *A. hydrophila*, *Pseudomonas*, and *Vibrio* was consistent during the 6-mo sampling period (Figs. 1, 2). The absence of *Vibrio* in June (Fig. 1) was caused by the accidental loss of cultures before identification was completed.

The total numbers of gut bacteria were consistently higher in striped bass from the Hudson River than in those from Long Island Sound (Fig. 3). Total gut flora ranged from 3.1×10^6 to 6.3×10^7/g for Hudson River fish and from 5×10^3 to 1.6×10^5/g for Long Island Sound fish. A general increase was noted in total gut flora in all samples from May through August, but the bacteria were consistently 100 to 1,000 times more abundant in estuarine than in marine samples.

Most kidney and liver tissues were without cultivable bacteria. In estuarine samples, *A. hydrophila* was found in three kidneys and two livers, and *Pseudomonas* in four kidneys and one liver. In coastal marine samples, *Micrococcus* occurred in four kidneys, and *A. hydrophila* and *Vibrio* in one liver each.

DISCUSSION

Except for the predominance of *A. hydrophila* the intestinal flora of striped bass was similar to that of most marine fishes: *Vibrio*, *Pseudomonas*, the *Achromobacter/Alcaligenes* group, and *Flavobacterium* are predominant (Shewan, 1961; Colwell, 1962; Aiso et al., 1968; Newman et al., 1972). The frequency of *A. hydrophila* in striped bass is similar to that found

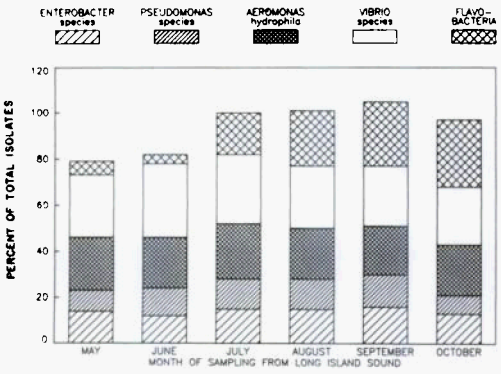

FIGURE 2. Monthly variation in the five most abundant intestinal bacteria in striped bass (*Morone saxatilis*) from a marine environment.

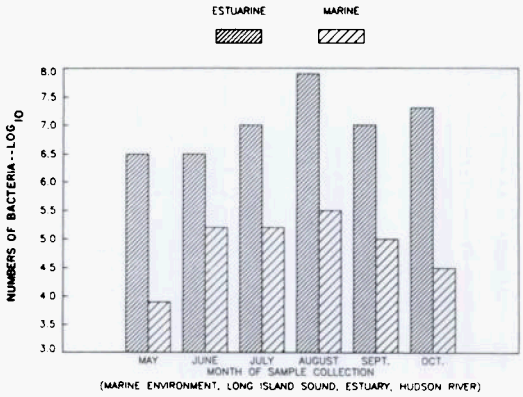

FIGURE 3. Monthly variation in intestinal bacteria in striped bass (*Morone saxatilis*) from marine and estuarine environments (Long Island Sound and Hudson River estuary).

by Allen and Pelczar (1967) in white perch (*Morone americanus*) in the Chesapeake Bay. The representative enteric bacteria in striped bass are also reflective of migrating fishes or marine fishes found in coastal waters (Newman et al., 1972; Horsley, 1973, 1977).

The predominance of the opportunistic pathogens *A. hydrophila*, *Pseudomonas*, and *Vibrio* in gut flora of striped bass in both estuarine and marine environments indicates the potential for epizootics if striped bass are stressed. The predominance of *Vibrio* species in the gut of striped bass from Long Island Sound is not surprising, because higher salinity favors the presence of this organism.

The consistently higher number of bacteria in gut flora of striped bass from the Hudson River might reflect the higher organic content in the lower Hudson River than in Long Island Sound. Observations in England, Germany, and the United States have indicated that the abundance of *A. hydrophila* depends on the organic load (Snieszko, 1983), and *A. hydrophila* was the predominant organism in the gut of Hudson River striped bass (Fig. 1, Table 1). Conversely, the finding of a higher percentage of flavobacteria in marine than in estuarine samples may be a result of the lower organic load. Compared with fish from polluted water, Horsley (1973) found that the numbers of flavobacteria were higher on the gills and skin of fish from water of low organic content (as well as in the water itself).

Other than assessments of sanitary conditions, only two studies of bacterial flora of fish from Long Island Sound have been reported; in both studies, the same groups of bacteria were found that had been reported in coastal waters by Murchelano and Brown (1970). These investigators found the numbers of *Pseudomonas*, the *Achromobacter/Alcaligenes* group, *Flavobacterium*, *Vibrio*, *Cytophaga*, *Micrococcus*, and *Bacillus* to be considerably different from those previously reported for coastal waters, and ascribed this difference to environmental diversity. The intestinal microflora of the striped bass isolated in our investigation included all of these genera except *Cytophaga*. However, *Cytophaga* may have been isolated from intestines if we had included Ordals medium (Anacker and Ordal, 1959) in this study. This medium was designed to demonstrate the creeping motility characteristic of *Cytophaga* and flexibacteria from fish.

Although the yellow pigmented flavobacteria are closely related to *Cytophaga*, isolates identified as *Flavobacterium* did

not show the gliding motility on agar medium characteristic of *Cytophaga*.

The qualitative makeup of the gut flora of striped bass in the Hudson River and Long Island Sound is similar to that found in other marine and estuarine fishes. However, the high percentage of known fish pathogens may predispose striped bass to bacterial epizootics, especially if populations are stressed by poor environmental conditions or by environmental contaminants.

ACKNOWLEDGMENTS

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WHY COPY EDITORS EXIST . . .

The following article appeared in the October 1983 issue of ASM NEWS and is reprinted here with permission from the American Society for Microbiology:

A recent president of ASM admitted that it took him 35 years to learn how to spell inoculate. The following list was submitted by Thomas D. Brock, who found it in the files of the late Elizabeth McCoy. These 73 imaginative spellings of inoculate and inoculation were compiled from more than 10,000 letters received from farmers in Wisconsin.

1. acknokalation
2. acknolate
3. annoxculate
4. anockkilacion
5. anoculating
6. auqulate
7. auqulating
8. enaculator
9. enocalation
10. enockolate
11. enoclating
12. enoculation
13. enonglation
14. inaucolation
15. inculating
16. inculocation
17. innocalate
18. innocculate
19. innockulating
20. innocqulate
21. innoculate
22. inocalation
23. inocalting
24. inocalton
25. inocolation
26. inocolulation
27. inocoulum
28. inocculating
29. inocklation
30. inoctulation
31. inoculatin
32. inonculating
33. inoculateon
34. inonculation
35. inoculin
36. inauguration
37. inocalate
38. inoculators
39. inoculant
40. inoculion
41. inonctolation
42. knockaliting
43. knockolacion
44. knoxalation
45. nockalate
46. nockate
47. nockelet
48. nockilation
49. nocklation
50. nocklate
51. noclate
52. noculate
53. noculation
54. nopelation
55. notiglation
56. noxcilating
57. noduletion
58. nuculate
59. enacalatation
60. enochalayion
61. anockolizing
62. innogelation
63. occulation
64. anackulaction
65. inacilat
66. enokilation
67. noxilation
68. nockulation
69. inockulation
70. nockalation
71. inocuilate
72. inagulatian
73. inoculatenlaction