QUANTITATIVE AND QUALITATIVE BACTERIOLOGY OF ELASMOBRANCH FISH FROM THE GULF OF MEXICO[1]

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ABSTRACT

Twelve species of elasmobranch fish (8 sharks, 2 rays, 1 skate, and 1 guitarfish) taken from the Gulf of Mexico off Sarasota, Florida, were studied. Numbers of bacteria on skin were recorded, as were types of bacteria occurring on skin, gills, teeth, and in intestinal contents. Comparative observations were made on eight species of osteichthyan fish and seawater. Counts/cm² of elasmobranch skin varied greatly both among genera and within a given species. In general, skin displayed relatively high counts which could be of significance in subsequent flesh spoilage. One brief study of spoilage of nurse shark meat at 5°C and room temperature (24°-26°C) showed that, after 7 days, species of *Pseudomonas, Vibrio*, and *Micrococcus* were dominant at the lower temperature while *Micrococcus* and *Proteus vulgaris* were recovered at 24°-26°C. Various types of bacteria found in or on the several areas of elasmobranch fish examined were compared with the little information available in the literature. Overall, Gram negative bacteria, particularly the genera *Pseudomonas* and *Vibrio*, were most common although several species of Gram positive bacteria were found also. *Planococcus* isolates from skin may represent important organisms because they have been implicated in shrimp spoilage. Three genera of hemolytic bacteria (*Proteus, Staphylococcus, Streptococcus*) were recovered from teeth of several elasmobranchs and may present a hazard to bite victims. Also, a variety of enteric bacteria potentially pathogenic to humans was found in intestinal contents; therefore, caution is suggested in handling shark material.

Considerable information is available on the normal and spoilage microflora of marine fish (e.g., Shewan 1961, 1971; Horsley 1977). However, the bacteriology of the elasmobranchs (sharks, skates, rays) is less understood despite a widespread present commercial fishery in local areas (Riedel 1961; McCormick et al. 1963) and its future potential (Juhl 1973; U.S. Department of Commerce 1982). Venkataramen and Sreenivasan (1955) studied the bacterial flora of skin of one shark caught off India; Johnson et al. (1968) characterized the intestinal microflora of five species of sharks obtained in the Indian Ocean; and Yap (1979) reported on skin isolates of two sharks freshly caught off Australia. Liston (1957) studied the bacteria associated with slime and gills of fresh North Sea skate. Spoilage bacteria in shark flesh were noted by Wood (1950) in Australia and Velankar and Kamasastri (1955) in India. Although the number of shark attacks on humans worldwide is statistically small (Baldridge 1974; Coppleson 1975), there are no substantive data on the potential bacteriological hazard of shark bites other than brief notations of hemolytic bacteria being recovered from the teeth of sharks (Davies 1960; Davies and Campbell 1962).

Consequently, this study was initiated to characterize the numbers and types of bacteria associated with a wide variety of elasmobranch fish common to the Gulf of Mexico. Comparative data were recorded for water and osteichthyan fish caught in the same area. These results will have relevance to the potential spoilage of elasmobranch meat and the pathobiology of shark bites.

METHODS

Sampling Sites

All fish were obtained from the Gulf of Mexico within several kilometers off Sarasota, Fla., or in the contiguous waters of Sarasota Bay. Small elasmobranchs were caught by use of a long, monofilament gill net set from the surface to a depth of about 1 m. Larger sharks were caught using baited longlines farther offshore. The one sand tiger shark, *Odontaspis taurus*, studied was obtained from the Mystic Marinelife Aquarium (Mystic, Conn.) and had been dead and refrigerated for 4 h. This shark, caught off the coast of New Jersey 3 d previously, was maintained in chlorinated brine water at the aquarium for 2 d

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prior to death. Only teeth and intestinal contents were sampled. For comparative bacteriological studies, several osteichthyan fish were caught by rod and reel or retrieved from the gill net mentioned above. Some elasmobranchs were occasionally maintained in large concrete or fiberglass tanks containing seawater piped from Sarasota Bay. All fish examined from tanks were either alive or had been dead for less than 1 h. Fish caught in the Gulf of Mexico were either iced if dead or kept in a wet hold until examined, which was routinely less than 1 h. In one case (see below) sharks were dead for 3 h before sampling. Water samples were collected from either Sarasota Bay or the tanks containing fish.

Quantitative Analysis

Swabbing was compared initially with two other methods involving the use of membrane filters in a quantitative sampling of elasmobranch skin. In the swabbing technique, a sterile aluminum foil template containing a 3.1 × 3.1 cm square opening (9.6 cm^2) was placed on the skin, on the side of each fish just posterior to the gills. A sterile polyester-tip swab (Falcon No. 2069[3]) was used over the exposed area in all directions, and the tip was broken off in a screw-capped tube containing 10 ml of sterile seawater. Decimal dilutions of this were prepared in 9 ml of sterile seawater and 0.1 ml volumes spread (Buck and Cleverdon 1960) on Bacto-Marine Agar (Difco Laboratories, Detroit, Mich.). One procedure with membrane filters involved placing sterile 0.45 μm gridded membranes (Millipore No. HAWG047SO) on shark skin and pressing them down by rolling a sterile glass rod across the membrane. The membrane was then placed grid uppermost on the surface of an agar plate. The second membrane filter technique was similar to the first except that the membrane, after exposure to the skin, was placed in a sterile plastic screw-cap centrifuge tube with sterile seawater and agitated on a vortex mixer for 30 s. Decimal dilutions and platings were then made as indicated above. All plates were incubated at room temperature (24°-26°C) for 3-5 d. Counts/cm^2 of skin were calculated.

Qualitative Analysis

In addition to skin, other body areas including teeth, gills, and intestinal contents were sampled by use of a swab. The upper third of plates of eosin-methylene blue, tryptic-soy, brain heart infusion, and marine agar (all Difco) was swabbed, and the remaining two portions of the plate were streaked sequentially with a sterile wire loop to isolate colonies. Plates were incubated at room temperature (24°-26°C) and 37°C for 1-5 d and colonies were selected based on differences in morphology.

Qualitative changes in the bacterial flora on shark flesh were assessed as a function of time and temperature. Pieces of nurse shark, *Ginglymostoma cirratum*, flesh (about 2 cm^2) were cut aseptically from one area of one side of the fish after the skin had been removed. These pieces were placed in sterile petri dishes and incubated at room temperature (24°-26°C) and 5°C. Initially and after 3, 4, 5, and 7 d incubation, the surface of the flesh was sampled using a sterile loop, which was used to directly inoculate agar plates to obtain well-isolated colonies.

Tank and bay samples were collected at a depth of about 30 cm in sterile bottles. One ml volumes were diluted in 9 ml of sterile seawater and additional decimal dilutions prepared in a similar manner. Spread plates (see above) were made on marine agar. Representatives of various colonial types were selected and identified after incubation for 3-5 d at room temperature.

All isolates were maintained on slants of either marine agar or tryptic-soy agar. Gram reactions were recorded by both conventional staining and the KOH technique (Buck 1982). Gram negative enteric bacteria were identified using either the Enterotube II (Roche Diagnostics, Nutley, N.J.) or API 20E (Analytab Products, Plainview, N.Y.) systems. Hemolysis was detected on tryptic-soy agar containing 5% horse blood. Other bacteria were characterized using the methods of Shewan et al. (1960) and Oliver (1982).

RESULTS AND DISCUSSION

Quantitative Analysis

Counts by the swab technique averaged 115% higher than those obtained by membrane filters applied directly to agar plates (two experiments) and 910% higher than counts by agitating the membrane in seawater followed by dilution and plating (three experiments). All subsequent counts of skin bacteria on both elasmobranch and osteichthyan fish were made using the swab

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

technique (Yap 1979), although this technique has inherent weaknesses (Horsley 1977), especially in examining shark skin, because it is abrasive and essentially three-dimensional. Nonetheless, counts obtained by swabbing a known area were always higher than those achieved by pressing a membrane filter on the skin, probably because the membrane did not recover bacteria associated with the lower portion of the denticles. The fibrous texture of the swab, while prone to some shredding unless care was used, may have allowed penetration into the skin. Perhaps an agar-coated slide or "paddle" which could be pressed onto shark skin would be more effective, although such a procedure might be unwieldy in the field.

Table 1 shows the number of bacteria recovered from the skin of various elasmobranch and osteichthyan fish. Elasmobranch skin showed a very wide range of counts both among genera and within a given species. Data in Table 1 indicated that there was no obvious correlation between bacterial counts on tank-held and freshly caught elasmobranchs, although bacterial counts on skin of three tank-held Atlantic sharpnose sharks were about two orders of magnitude lower than counts for one specimen of the same species caught in a gill net. Three of the highest counts on shark skin noted in Table 1 (bonnethead, 410,000/cm^2; blacktip, 530,000/cm^2; blacknose, 330,000/cm^2) were from fish which had been dead for 3 h. This suggests that bacteria rapidly colonize skin of dead sharks. Yap (1979) reported varying counts on skin of freshly caught shark which depended on the area of the fish sampled. His counts (310-1,900/cm^2) were, in general, lower than those reported herein, and were estimated from broth dilutions and not plates. The shark skin sampled here displayed relatively high bacterial counts which are of considerable significance if the sharks are to be used for food. Large numbers of bacteria could be deposited onto flesh which subsequently may undergo more rapid spoilage if not adequately washed and/or refrigerated. With the exception of the bighead searobin, *Prionotus tribulus*, the counts on osteichthyan fish were quite similar and within the range reported in other studies (Horsley 1977).

TABLE 1.—Number of bacteria on fish skin (Bacto-marine agar).

Taxon	Source	No./cm^2
Elasmobranchs		
Florida smoothhound, *Mustelus norrisi*	Gill netted	[1]120
		[1]840
Nurse shark, *Ginglymostoma cirratum*	Gill netted	[1]8,200
Atlantic sharpnose shark, *Rhizoprionodon terraenovae*	Gill netted	[1]2,300
		220,000
		[1]1,000
		[1]1,000
Bonnethead or shovelhead shark, *Sphyrna tiburo*	Gill netted	400,000
		42,000
		1,100
		410,000
Brown or sandbar shark, *Carcharhinus plumbeus*	Longline caught	2,300
Blacktip shark, *Carcharhinus limbatus*	Gill netted	530,000
Blacknose shark, *Carcharhinus acronotus*	Gill netted	330,000
Tiger shark, *Galeocerdo cuvieri*	Longline caught	240
Atlantic guitarfish, *Rhinobatos lentiginosus*	Gill netted	[1]260
		[1]460
Clearnose skate, *Raja eglanteria*	Gill netted	[1]100,000
		80,000
Southern stingray, *Dasyatis americana*	Gill netted	[1]6,700
Cownose ray, *Rhinoptera bonasus*	Gill netted	50,000
Osteichthyes		
Gulf menhaden, *Brevoortia patronus*	Gill netted	23,000
Southern flounder, *Paralichthys lethostigma*	Rod caught	26,000
Spanish mackerel, *Scomberomorus maculatus*	Gill netted	15,000
Ladyfish, *Elops saurus*	Gill netted	19,000
Searobin, *Prionotus tribulus*	Gill netted	100
Black drum, *Pogonias cromis*	Gill netted	15,000
Permit, *Trachinotus falcatus*	Gill netted	7,100
Atlantic spadefish, *Chaetodipterus faber*	Gill netted	14,000

[1]Tank held.

Qualitative Analysis

Table 2 shows the number of isolates and genera of bacteria recovered from elasmobranch and osteichthyan fish and from waters where the fish were taken or held. The Gram negative bacteria, especially *Pseudomonas, Vibrio*, and *Cytophaga*, accounted for 89% of the 111 isolates from skin. In other studies, pigmented Gram positive isolates of *Micrococcus*, *Bacillus*, and *Corynebacterium* were the most common (30 strains) on the skin of one shark (*Carcharhinus* sp.) caught off India; only 5 cultures of Gram negative *Achromobacter, Flavobacterium*, and *Vibrio* were recovered (Venkataraman and Sreenivasan 1955). *Pseudomonas* (40%), Micrococcaceae (30%), and *Moraxella* (15%) were dominant on skin of a freshly caught shark off Australia (Yap 1979). The data here for skin (Table 2) show a similar percentage for *Pseudomonas* but considerably fewer isolates of *Moraxella* and Gram positive cocci.

Table 2 shows that for Gulf of Mexico sharks, fewer genera were recovered from intestines than from other areas, but that the Gram negative genera were predominant and species of *Photobacterium, Pseudomonas*, and *Vibrio* accounted for 57% of the isolates. Gram positive bacteria (one

TABLE 2.—Number of isolates and percentage of bacterial genera recovered from elasmobranch and osteichthyan fish and water of the Gulf of Mexico.

	Elasmobranchs								All elasmobranch samples		Osteichthyes skin		Water	
	Skin		Gills		Teeth		Intestines							
Genus	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Gram negative														
Aeromonas	1	1			2	3			3	1			2	4
Acinetobacter	4	4	2	10	6	8	1	7	13	6	3	7		
Alcaligenes	2	2	1	5					3	1	2	5	4	8
Cytophaga	11	10	1	5	1	1	1	7	14	6	1	2		
Flavobacterium	1	1			4	5	1	7	6	3	1	2		
Flexibacter	1	1							1	1				
Moraxella	4	4			1	1			5	2	2	5	3	6
Photobacterium	4	4	4	18	5	7	4	29	17	8			6	12
Pseudomonas	35	32	1	5	10	13	2	14	48	22	8	18	15	30
Vibrio	30	27	10	46	17	23	2	14	59	27	16	36	15	30
Xanthomonas	6	5	1	5	2	3			9	4	4	9	2	4
Gram positive														
Arthrobacter	4	4			3	4	1	7	8	4			1	2
Bacillus					4	5	1	7	5	2	4	9	1	2
coryneforms	1	1	1	5	1	1			3	1	2	5	1	2
Micrococcus-Staphylococcus	4	4	1	5	8	11			13	6	2	5	1	2
Planococcus	3	3			2	3			5	2				
Streptococcus					9	12	1	7	10	5				
Total	111		22		75		14		222		45		51	

isolate each of *Arthrobacter, Bacillus*, and *Streptococcus*) represented 21% of the total. In a study of intestinal material from five species of sharks caught in the Indian Ocean, 10 isolates of *Bacillus* were found, and 1 each of *Corynebacterium, Alcaligenes, Vibrio, Spirillum*, and *Xanthomonas*; one animal showed no bacteria (Johnson et al. 1968).

No data are available in the literature on bacterial types recovered from shark gills, although the gills and skin of North Sea skates have been studied (Liston 1957). Gram negative bacteria were dominant with *Pseudomonas* most common on both skin and gills. Qualitative observations of skin agreed with the present data (Table 2), but skate gills showed a much higher percentage of *Pseudomonas* (60%) compared with this study (5%). The other Gram negative bacteria from skate gills were also found in this study (Table 2).

Hemolytic bacteria were isolated from the teeth of sharks in the present study. *Streptococcus* spp. were recovered from teeth of shovelhead, *Sphyrna tiburo*, and sand tiger, *Odontaspis taurus*, sharks; *Staphylococcus* spp. were found on the teeth of a cownose ray, *Rhinoptera bonasus*; and *Providencia rettgeri* was recovered from teeth of two shovelhead sharks. All of these bacteria were from sharks taken in the Gulf of Mexico except for the sand tiger shark which was caught off New Jersey and had been in captivity for only 3 d. In addition, several hemolytic species of *Vibrio* have been isolated recently from the teeth of a white shark, *Carcharodon carcharias*, caught off Block Island, R.I. (Buck et al. unpubl. data[4]).

Hemolytic bacteria were found in the mouths of sharks from South African waters, and it was suggested that bacterial infections of bites could have been a contributing factor in the deaths of victims (Davies 1960). The hemolytic bacterium recovered from teeth of *Carcharhinus zambezensis* (*leucas*?) was described as a "Paracolon bacillus" (Davies and Campbell 1962).

The present observations not only confirm the occurrence of hemolytic organisms on teeth of sharks in nature but also extend these types to include bacteria not reported previously from sharks and the number of species of sharks which harbor them. They suggest that shark bites could possibly introduce potentially pathogenic bacteria into the tissues of victims.

A variety of enteric bacteria was found associated with the intestinal contents and occasionally the teeth of elasmobranchs; none were recovered from the gills or skin. These data are presented in Table 3. Three cultures only, all *Shigella* species, were isolated from bony fish. One was found in pinfish, *Lagodon rhomboides*, intestine, and two strains were isolated from a black drum, *Pogonias cromis*—one on the gills and the other from intestinal contents.

[4]Buck, J. D., S. Spotte, and J. J. Gadbaw, Jr. Manuscr. in prep. Bacteriology of the teeth from "Jaws": Medical implications for shark-bite victims.

Enterobacteria are found frequently on osteichthyan fish, but there are no reports in the literature on their occurrence in (on) elasmobranchs. If waters contain domestic wastes, then the fish will almost certainly be contaminated also (Shewan 1971; Horsley 1977). Coliform counts in Sarasota Bay are generally low, although counts of 1,800/100 ml have been recorded in one bayou receiving treated sewage effluent (Buck, unpubl. data[5]). Areas north (Tamplin et al. 1982) and south (Peterson and Yokel 1983) of Sarasota Bay have shown the presence of potentially pathogenic enteric bacteria. Consequently, the elasmobranch fish studied here may well have been in contact with sources of enterobacteria. The enteric bacteria encountered on the teeth and in the intestines of several elasmobranchs probably reflected feeding habits and originated on smaller prey which had passed through waters receiving human and/or animal excretions. Enteric bacteria do not multiply in passage through rainbow trout but temperature may be an important factor (Lesel and Peringer 1981; Lesel and LeGac 1983). The internal temperature of some sharks (Lamnidae) (Carey et al. 1981; Smigh and Rhodes 1983) is significantly warmer than the surrounding water. In subtropical areas, increased water temperature and that of the interior tissues of elasmobranchs might provide an environment that encourages bacterial multiplication, including potential pathogens. While none of the enterobacteria, except perhaps *Shigella* species, recovered from intestines and teeth of elasmobranchs represent primary pathogens, members of the other genera are commonly found as secondary or opportunistic pathogens in humans. Thus, caution should be exercised when handling dead shark material, particularly internal organs such as the digestive tract.

The genera *Vibrio* and *Pseudomonas* were predominant bacteria in combined data for all elasmobranch samples (Table 2). When isolates for tank-held and open-water fish were compared, these two genera were the most common in each group. The occurrence of other microbes did not vary more than 6% for any genus of bacteria between tank-held and freshly caught elasmobranchs, except for *Photobacterium* species which represented 11% of the isolates from the former and 3% of the latter.

The bacterial flora of osteichthyan fish and seawater consisted largely of Gram negative bacteria (82% and 94%, respectively), with *Vibrio* and *Pseudomonas* predominating. No substantial differences in generic composition were noted between Sarasota Bay water and fish holding tanks. Fewer numbers of several other Gram negative forms were found; these results agree with those of others (e.g., Shewan 1961). Small populations of Gram positive bacteria (*Arthrobacter, Bacillus*, cocci) were noted and probably represented terrestrial influence because the fish were taken from nearshore waters. This assumption may require reevaluation because there may be a widespread distribution of Gram positive bacteria in seawater (Gunn et al. 1982).

The microflora of spoiling shark muscle (no species indicated) from Australia have been studied, and the genus *Corynebacterium* was the dominant organism; *Pseudomonas* species and Gram positive cocci were also found in large numbers (Wood 1950). Few coryneforms were isolated in the present study, although *Pseudomonas* and Gram positive organisms were commonly recovered. In the brief study here of nurse shark flesh, the dominant bacteria found initially were species of *Vibrio* and *Pseudomonas*. After 7 d of incubation at 5°C, the flora were composed principally of *Pseudomonas, Vibrio*, and *Micrococcus*. When flesh was held at room temperature (24°-26°C), Gram positive cocci and *Proteus vulgaris* were

[5]J. D. Buck, University of Connecticut Marine Research Laboratory, Noank, Conn., unpubl. data, 1982.

TABLE 3.—Enterobacteriaceae isolated from elasmobranch fish.

Taxon	Bacteria
Nurse shark, *Ginglymostoma cirratum*[1]	*Proteus vulgaris*
Intestine	*Escherichia coli*
Shovelhead shark, *Sphyrna tiburo*	
Intestine	*Enterobacter agglomerans*
	Escherichia coli
	Shigella sp.
Teeth	*Citrobacter freundii*
	Providencia rettgeri
	Providencia sp.
Sandbar shark, *Carcharhinus plumbeus*	
Intestine	*Shigella* sp.
Teeth	*Proteus vulgaris*
	Providencia rettgeri
Blacktip shark, *Carcharhinus limbatus*	
Intestine	*Escherichia coli*
	Providencia alcalifaciens
	Shigella sp.
Teeth	*Escherichia coli*
	Proteus vulgaris
Sand tiger shark, *Odontaspis taurus*[1]	
Intestine	*Citrobacter freundii*
	Morganella morganii
	Proteus vulgaris
Cownose ray, *Rhinoptera bonasus*	
Intestine	*Shigella* sp.
Teeth	*Serratia liquefaciens*
Clearnose skate, *Raja eglantera*[1]	
Intestine	*Escherichia coli*

[1]Tank held.

predominant after 7 d. The latter is capable of hydrolyzing urea, and several species of *Micrococcus* are urease-positive (Buchanan and Gibbons 1974); hence, both of these groups are potential contributors to shark tissue spoilage. This enrichment of Gram positive types in elasmobranch spoilage was noted by Wood (1950).

Bacteria were found in 12 samples of shark muscle (*Scoliodon* sp.) allowed to spoil at 27°-30°C (Velankar and Kamasastri 1955). No coryneforms and only one *Micrococcus* isolate were found; all others were unidentified Gram negative nonpigmented rods.

The spoilage of iced abdominal wall muscle of Australian school shark, *Galeorhinus australis*, was studied by Yap (1979). *Pseudomonas* and *Moraxella* (45% and 20%, respectively) were the dominant bacteria recovered after 10 d although the Gram positive cocci represented 15% of the total.

The data presented here for the flesh spoilage experiment, albeit limited, confirm the observations of Wood (1950) and Yap (1979), but none of these parallel the findings of Velenkar and Kamasastri (1955) which also concerned sharks from subtropical waters. Perhaps the local marine microflora or experimental conditions influenced their observations.

Although the number of isolations was relatively small, the genus *Planococcus* was found associated with elasmobranch skin and teeth in this study. All the cultures recovered were yellow-pigmented and were probably *Planococcus citreus*, the only accepted species (Buchanan and Gibbons 1974). This proteolytic bacterium has been implicated in shrimp spoilage (Alvarez 1982) and may be a significant spoilage organism of elasmobranch flesh.

CONCLUSIONS

The observations reported here have shown that elasmobranch fish contain a large and diverse bacterial flora. Because there is little information on the microbiology of sharks, skates, and rays, assessing the relative significance of the data is difficult. In many cases, counts of bacteria on the skin were an order of magnitude higher than those noted on osteichthyan fish caught in the same waters. In other samples, counts were two orders of magnitude lower. Considerable variation was seen in individual species of elasmobranchs. Types of bacteria recovered from different areas of fresh fish and during one controlled spoilage experiment on flesh did not correlate well in all respects with results of other studies which in some instances were limited to one or a few fish or different species than those considered here. Also, little information was provided in the literature on cultural conditions and other variables which could affect development of various bacteria reported. The data here substantiate the occurrence of certain potential spoilage bacteria on skin and include the genus *Planococcus* which has been implicated in shrimp spoilage. The present study also confirms and extends other observations on the occurrence of hemolytic bacteria on shark teeth. In addition, potentially pathogenic enterobacteria were recovered from teeth and intestinal contents of several elasmobranch species. It is hoped that future studies will include larger numbers of additional shark species for a clearer assessment of the role of bacteria in both spoilage and public health aspects of a valuable and underutilized marine resource.

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