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[Article]

Blood Culture Results from Healthy Captive and Free-Ranging Elasmobranchs

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Abstract.—Blood culture is a diagnostic tool used in confirming bacterial disease in teleostean and elasmobranch fishes. Unlike teleosts, elasmobranchs have a normal microflora in multiple organs, but their blood has generally been considered to be sterile. In regular exams of elasmobranchs conducted at a public aquarium, occasional blood samples have tested positive on culture. This finding prompted a blood culture survey of healthy captive and wild elasmobranchs (sharks and stingrays), which showed that 26.7% of all animals were positive. Stingrays alone showed a 50% occurrence of positive blood cultures, although the total number of animals was low and freshwater species were included in this number. When elasmobranchs other than stingrays were evaluated according to metabolic category, pelagic animals had a higher percentage of positive cultures than nonpelagic animals (38.7% versus 13.9%). These results indicate that a single positive blood culture without other corroborating diagnostics is not sufficient to confirm septicemia in elasmobranchs.

Blood cultures are standard diagnostic tools for confirming bacteremia and septicemia in clinically ill animals (Nostrandt 1990; Walker 2004). There are numerous reports of positive blood culture results corroborating disease in many species including dogs (Hirsh et al. 1984; Nostrandt 1990), cats (Nostrandt 1990), cows (Cullor 1992; Hariharan et al. 1992; Fecteau et al. 1997), horses (Wilson and Madigan 1989; Hariharan et al. 1992; Marsh and Palmer 2001), reptiles (Novak and Seigel 1986; Work et al. 2003), amphibians (Olson et al. 1992), and teleostean fishes (DeGuzman and Shotts 1988). Bacteremia is caused by vascular invasion from a primary site of infection (chronic dental disease, endocarditis, enteritis, or an abscess) or iatrogenic sources (catheter placement, dental manipulation, or surgery) (Reimer et al. 1997; Beers and Berkow 1999).

Traditionally, blood cultures have been performed on fish during postmortem examinations, and they have been recommended as a method of nonlethal testing for pathogens and systemic disease (Stoskopf 1993a; Francis-Floyd 1999; Klinger et al. 2003). Such a test is particularly appealing because fish frequently fail to manifest clear clinical signs with early systemic bacterial infections, instead displaying nonspecific signs such as inappetance and lethargy. As these diseases progress towards septicemia more obvious signs like hyperemia, petechiae, and dermal hemorrhages appear (Noga 1999), yet these signs are very general and may be indicative of other infectious and noninfectious etiologies. In general, animals at this late stage are less likely to respond to treatment. Early detection of these diseases can lead to positive clinical outcomes with medical treatment. Blood cultures may also serve as an indicator of disease risk with potential pathogens (e.g., vibriosis) to conspecific animals living in the same environment as their infected counterparts.

The study of infectious disease in elasmobranch fishes has not been extensive. Bacterial disease has been reported in sharks and stingrays with *Vibrio* spp. the most commonly identified etiologic agent (Stoskopf 1993b; Pedersen et al. 1997; Terrell 2004). Other agents identified in systemic bacterial diseases of sharks include *Citrobacter freundii*, *Corynebacterium pseudointeriticum*, *Micrococcus* (A. George, University of Rhode Island, unpublished), *Aeromonas salmonicida* (Briones et al. 1998), *Streptococcus* Lancefield group B (W. H. Carr et al., North Carolina State University, unpublished), and *Streptococcus acidominus* (P. D. Govett et al., unpublished). In some of these cases blood cultures were used as an antemortem diagnostic tool, but most of the diagnoses were obtained on postmortem examination using multiple tissue sources for culture with corroborating gross and histopathological results.

Early microbiological studies with elasmobranch tissues were conducted to evaluate the spoilage of

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carcasses intended for human consumption (Wood 1950). Examination of muscle tissue and other organs showed that shark tissues had a high degree of recoverable microflora within various organ tissues. One study showed that sharks have autochthonous flora within numerous organs including intestine, liver, spleen, kidney, pancreas, and on teeth and skin (Grimes et al. 1985). Other investigations have indicated that elasmobranch blood (collected both antemortem as well as postmortem) is typically sterile although sample sizes were low (Grimes et al. 1985; Knight et al. 1988; Cheung and Schneider 1990). A widely accepted view in veterinary medicine is that blood is sterile in normal animals, even though baseline studies have not been performed in most species. Reports of normal transient bacteremias and positive blood cultures in otherwise healthy animals have rarely been published (Hanel et al. 1999).

Recent regular examinations at a public aquarium noted occasional positive blood cultures in healthy animals, prompting this prospective study using standard veterinary techniques to assess the presence or lack of bacteria in the blood of a variety of elasmobranchs. Some samples were drawn during routine physical exams of a captive population of elasmobranchs and others were collected opportunistically among free-ranging animals.

Methods

Fish.—Captive adult sharks and stingrays of both sexes housed at the John G. Shedd Aquarium, Chicago, Illinois, were immobilized for annual health screens using 100–125 mg/L tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, Washington). The animals were deemed healthy based on normal appearance, appetites, findings from physical examination, hematologic and serum chemical analysis, and rapid (<10 min) recoveries from anesthesia. The study included individuals of the following species (the sex ratio is represented as the number of males, the number of females, and the number of unknown sex, the numbers being separated by periods): the spotted eagle ray *Aetobatus narinari* (0.1.0), blackfin reef shark *Carcharhinus melanopterus* (13.1.1), sandbar shark *C. plumbeus* (3.1.1), swell shark *Cephaloscyllium ventriosum* (1.1.0), whitespotted bamboo shark *Chiloscyllium plagiosum* (8.5.0), horn shark *Heterodontus francisci* (2.1.1), mangrove whiptail ray *Himantura granulata* (2.2.0), bearded shark (also known as Japanese wobbegong) *Orectolobus japonicus* (2.2.0), freshwater stingrays *Potamotrygon* spp. (1.1.0) and tiger ray *P. menchacai* (1.0.0), narrowsnout sawfish (also known as green sawfish) *Pristis zijsron* (0.1.0), shovelnose guitarfish *Rhinobatos productus* (0.0.1), variegated shark *Stegastoma varium* (also known as zebra shark *S. fasciatum*; 4.4.0), whitetip reef shark *Triaenodon obesus* (2.12.0), and leopard shark *Triakis semifasciata* (2.1.0). Some individuals were sampled repeatedly. Water quality values in systems housing study fish were considered within normal limits for elasmobranchs (Mohan and Aiken 2004). Environmental cultures were not routinely performed in systems that were considered stable based on water quality values and fish health.

Additionally, free-ranging subadult sharks were sampled opportunistically during manual restraint for hook removal. Individual sharks in this group were hook-and-line caught during a normal collecting trip in the coastal waters of southern Florida 35 km east of Marathon Key. Individuals of the following species were included: the blacknose shark *Carcharhinus acronotus* (2.1.6), blacktip shark *C. limbatus* (0.5.0), nurse shark *Ginglymostoma cirratum* (0.3.0), lemon shark *Negaprion brevirostris* (1.0.0), and Atlantic sharpnose shark *Rhizoprionodon terraenovae* (1.0.0).

Sample collection.—Skin was cultured before and after disinfection in 31 captive sharks, including the blackfin reef shark (13.1.0), sandbar shark (1.2.0), bearded shark (2.1.0), variegated shark (3.2.0), and whitetip reef shark (1.8.0) to assess the effectiveness of the techniques that were used to prepare the skin before blood collection. Culturette swabs (BBL, Becton Dickinson, Sparks, Maryland) were wiped across the skin at the midline ventral tail where blood collection was planned; one swab was taken before disinfection and one swab after. Disinfection consisted of one swipe of an isopropyl alcohol pad (Triad Disposables, Brookfield, Wisconsin) over the intended venipuncture area; after the swipe, the alcohol was allowed to sit for 1 min before resampling. Only one swipe was performed because vigorously rubbing elasmobranch skin with disinfecting agents has been observed to cause a significant dermatitis with a long healing time (N.D.M., observation).

Blood was aseptically collected from 195 elasmobranchs of the following species: spotted eagle ray (0.1.0), blacknose shark (2.1.6), blacktip shark (0.5.0), blackfin reef shark (44.2.1), sandbar shark (13.3.1), swell shark (2.2.0), whitespotted bamboo shark (11.7.0), nurse shark (0.3.0), horn shark (4.2.2), mangrove whiptail ray (2.2.0), lemon shark (1.0.0), bearded shark (4.9.0), *Potamotrygon* spp. (1.1.0), tiger ray (1.0.0), narrowsnout sawfish (0.3.0), shovelnose guitarfish (0.0.1), Atlantic sharpnose shark (1.0.0), variegated shark (11.7.0), whitetip reef shark (9.21.0), and leopard shark (2.5.0). The samples were collected after disinfection by inserting a needle at the ventral midline of the tail and advancing until a vertebra was

reached and a blood flash noted. Samples were drawn using a variety of needle (18–22 gauge, 3.8 cm) and syringe sizes (5–12 mL) appropriate for the size and shape of the animal. Usually, 10 mL of blood was drawn from each animal to provide a sufficient sample for additional diagnostic testing. After the syringe apparatus was withdrawn, the needle was discarded, a new needle was inserted onto the syringe hub, and 1 mL of blood was injected into special media for blood cultures as described in the next section. Samples from the free-ranging fish were placed on ice immediately after collection and shipped overnight to the laboratory.

Microbial culture and identification.—Skin culture swabs were processed within 1 h of collection. Each sample was plated on a blood agar and a thiosulfate citrate bile salts sucrose (TCBS) agar plate (Remel, Lenexa, Kansas) and incubated at 35°C. Cultures were observed for growth at 24 h and again at 48 h. Each organism isolated was subjectively categorized as having heavy, moderate, light, or rare growth. Isolates were replated for purity and amplification.

One-milliliter samples of aseptically collected blood were inoculated into Septi-Chek BHI pediatric, 20-mL-volume bottles (BBL, Sparks, Maryland) and incubated at 35°C. All samples were processed within 1 h of collection except for those from free-ranging fish, which were shipped on ice overnight then incubated at 35°C on arrival. Cultures were assessed daily for 7 d. Cultures were deemed negative if there was no growth by the seventh day. At any indication of turbidity in the media during incubation, a few drops were removed with a tuberculin syringe and the sample was streaked on a blood agar plate (Remel). After overnight incubation at 35°C the plates were checked for growth.

Isolates were screened with oxidase, spot indol, and catalase and incubated to a triple sugar iron (TSI) slant (Remel) and glucose oxidation/fermentation media tubes (Remel). Identification was performed using API 20E or API NE strips (Biomerieux, Durham, North Carolina). The coagulase reaction was used to screen for gram-positive cocci. Coryneforms were reported based on gram stain appearance.

Animals were categorized by metabolic categories as pelagic or nonpelagic to determine whether there were differences in culture results between lifestyle patterns. Pelagic fish swim continuously to provide a constant water flow over the gills (ram-jet ventilation) and have a high requirement for oxygen, whereas nonpelagic fish are capable of resting on the seafloor, actively pumping water over their gills and having a higher tolerance for low-oxygen conditions (Powell et al. 2004). Stingrays were considered nonpelagic elasmobranchs, but the pelagic spotted eagle ray was placed in this category because it is capable of pumping water over the gills and can rest on the seafloor.

Statistical analysis.—Data tabulation and minor calculations were performed in Microsoft Excel 2002 SP3 (Microsoft Corporation, Redmond, Washington). Chi-square analysis was performed using a computer-based online program (www.georgetown.edu/faculty/ballc/webtools/web_chi.html; accessed on February 24, 2007).

Results

Skin cultures were taken from 31 captive sharks before disinfection (Table 1). A total of 97 microorganisms were isolated from these swabs. All post-disinfection cultures were negative. *Photobacterium damsela* (16.5% [16 of 97]), *Vibrio alginolyticus* (11.3% [11 of 97]), and *Staphylococcus epidermidis* (10.3% [10 of 97]), respectively, were the organisms most commonly isolated.

Blood cultures were performed on 195 sharks and stingrays. Generally there was little growth of organisms at 24 h. If turbidity was noted in blood cultures it typically occurred after 72 h. There were positive results in 26.7% (52 of 195) of the total population that was sampled (Table 2). *P. damsela* (19.7% [12 of 61]), *S. epidermidis* (13.1% [8 of 61]), and *V. alginolyticus* (9.8% [6 of 61]), respectively, were the organisms most commonly isolated.

Separation of shark species into general taxonomic categories indicated 21% (38 of 181) positive blood cultures, while stingrays showed 50% (7 of 14) positive blood cultures. Pelagic fish (Table 3) showed a higher prevalence of positive blood cultures (38.7%) than their sedentary counterparts (18.3%). Chi-square analysis of these data showed a significant difference ($\chi^2 = 10.13$; $P < 0.01$). When the stingray data were eliminated and only sharks were compared, the difference between pelagic animals and sedentary animals was greater, 38.7% versus 13.9%. Chi-square analysis of these data showed a significant difference ($\chi^2 = 14.80$; $P < 0.001$).

Discussion

An apparently normal microflora in the blood of elasmobranchs was noted during routine health examinations on a captive population. Owing to the lack of any baseline studies, we conducted a prospective study to evaluate the presence or absence of bacteremia. In this study, fish were chosen only if they were healthy and habituated to captivity or were free-ranging fish sampled within minutes of rapid capture. Approximately 27% of the total fish sampled were blood-culture positive. While these data represent a wide diversity of species, they do show that there is a normal

TABLE 1.—Aerobic bacteria cultured from the skin of captive elasmobranchs before disinfection.

Microorganism	*N* by species	Total *N*
Acinetobacter junii/johnsonii	1[a]	1
Aeromonas sobria	3[b]	3
Brevundimonas vesicularis	1[a]	1
Chryseobacterium indologenes	1[c]	1
Chryseomonas luteola	4[b]	4
Citrobacter youngae	4[b]	4
Comamonas testosteroni/Pseudomonas alcaligenes	1[b], 1[a]	2
Coryneforms	1[b], 1[a]	2
Micrococcus spp.	1[a], 1[d]	2
Moraxella spp.	1[c], 2[a], 4[d]	7
Morganella morganii	1[b]	1
Ochrobactrum anthropi	1[b]	1
Pasteurella haemolytica	2[b], 1[e], 1[a], 3[d]	7
Pasteurella multocida	1[d]	1
Pasteurella pneumotropica	1[b]	1
Pasteurella pneumotropica/haemolytica	1[a]	1
Photobacterium damsela	5[b], 2[e], 2[a], 2[c], 5[d]	16
Proteus mirabilis	1[a]	1
Pseudomonas aeruginosa	1[e], 3[c], 1[d]	5
Pseudomonas fluorescens	1[c]	1
Pseudomonas putida	1[d]	1
Pseudomonas stutzen	1[a], 1[d]	2
Ralstonia spp.	1[a]	1
Staphylococcus epidermidis	5[b], 2[a], 3[d]	10
Streptococcus alpha	4[b]	4
Vibrio alginolyticus	3[b], 2[e], 3[a], 1[c], 2[d]	11
Vibrio fluvialis	1[b]	1
Vibrio parahaemolyticus	3[b]	3
Vibrio vulnificus	1[b], 1[a]	2

[a] Bearded shark ($N = 3$).
[b] Blackfin reef shark ($N = 14$).
[c] Variegated shark ($N = 5$).
[d] Whitetip reef shark ($N = 9$).
[e] Sandbar shark ($N = 3$).

bacteremia that can occur in elasmobranch fishes. Increasing the sample number per species would be ideal to further evaluate inter-specific differences. Some of the identified organisms are considered to be opportunistic pathogens in teleosts. These include *Aeromonas* spp., *Pseudomonas* spp., and *Vibrio* spp., which are present in the water and on healthy fish skin. Fish often succumb to these pathogens when faced with other exogenous or endogenous stressors such as poor environmental quality, poor nutrition, or a break in the integument (DeGuzman and Shotts 1988). This has also been noted in elasmobranchs where normal opportunistic flora becomes pathogenic under conditions of stress or concurrent disease (Grimes et al. 1984, 1986; Bertone et al. 1996; Pedersen et al. 1997).

Bacterial disease in fish is identified when positive culture results are obtained from internal tissues such as the blood or the pronephros (Henley and Lewis 1976; DeGuzman and Shotts 1988; Klinger et al. 2003). These cultures are often corroborated postmortem with the same organism in other organ cultures or by supporting pathology results. Several publications note that blood culture can be used as a nonlethal diagnostic tool in bacterial septicemia of fishes (Stoskopf 1993a; Francis-Floyd 1999; Klinger et al. 2003). Klinger et al. (2003) described a case study in which 9 of 20 sick animals were culture positive antemortem. In that study positive cultures were verified via postmortem examination of animals that succumbed to disease or were euthanatized. Blood culture has been used as an antemortem diagnostic tool in sharks for the detection of bacterial infection (Grimes et al. 1985; Cheung and Schneider 1990). The sample sizes for these evaluations were small and it is possible that the cultures were not incubated for a sufficient length of time for proper evaluation. Nevertheless, it has been generally accepted that blood is sterile in normal sharks (Grimes et al. 1985; Knight et al. 1988). This concept is supported throughout the veterinary literature where it is generally accepted that the circulatory system and the lymphoid tissues of animals do not have a normal microbial flora (Walker 2004).

Contamination of blood cultures has been extensively evaluated in the human medical literature, which indicates a 3–5% contamination rate (Reimer et al. 1997; Weinbaum et al. 1997). In any blood culture process the most obvious source of contamination would be the skin and entry site of the needle. To

TABLE 2.—Aerobic bacteria cultured from the blood of captive and wild elasmobranchs.

Microorganism	*N* by species	Total *N*
Aeromonas hydrophila caviae	1[a]	1
Alcaligenes spp.	1[a]	1
Chryseomonas luteola	2[a]	2
Citrobacter freundii	2[a]	2
Citrobacter youngae	3[a]	3
Moraxella spp.	1[b]	1
Morganella morganii	1[a], 1[c]	2
Pasteurella pneumotropica	1[a], 1[d], 1[b]	3
Photobacterium damsela	1[e], 7[a], 1[c], 1[f], 2[b]	12
Plesiomonas shigelloides	1[g]	1
Proteus vulgaris	1[c], 1[b]	2
Pseudomonas aeruginosa	1[a]	1
Pseudomonas alcaligenes	1[a]	1
Pseudomonas fluorescens	1[a]	1
Pseudomonas fluorescens/putida	1[g]	1
Pseudomonas stutzeri	1[a]	1
Shewanella putrefaciens	2[a], 1[b]	3
Sphingomonas paucimobilis	1[a]	1
Staphylococcus epidermidis	2[h], 2[a], 1[i], 1[j], 1[g], 1[b]	8
Stenotrophomonas maltophilia	2[j]	2
Streptococcus group D	1[b]	1
Vibrio alginolyticus	1[e], 1[a], 1[k], 2[c], 1[l]	6
Vibrio fluvialis	1[g]	1
Vibrio parahaemolyticus	1[e]	1
Vibrio vulgaris	1[c]	1
Vibrio vulnificus	1[i], 1[k]	2

[a] Blackfin reef shark.
[b] Whitetip reef shark.
[c] Mangrove whiptail ray.
[d] Narrowsnout sawfish.
[e] Blacktip shark.
[f] Bearded shark.
[g] *Potamotrygon* spp.
[h] Blacknose shark.
[i] Sandbar shark.
[j] Swell shark.
[k] Whitespotted bamboo shark.
[l] Leopard shark.

evaluate this possible source of contamination with the elasmobranchs in this study, skin cultures were taken before and after disinfection. This also served to evaluate the method of disinfection chosen, which involved one firm swipe of an alcohol swab across the skin at the intended venipuncture site. While a more rigorous disinfection method is employed with most taxa, elasmobranchs show an extreme sensitivity to common disinfectants and to vigorous rubbing of the skin. We have shown a 100% negative culture rate by gently removing the biofilm with an alcohol-soaked gauze over the intended venipuncture site. The skin organisms cultured (Table 1) before disinfection were all considered to be normal flora. Many of these organisms match other reports of microflora from elasmobranch skin (Wood 1950; Venkataraman and Sreenivasan 1955; Horsley 1977; Grimes et al. 1985; Cho et al. 2004).

TABLE 3.—Aerobic blood culture results of elasmobranchs categorized according to taxonomy (including or excluding stingrays) and locomotive–respiratory life history (pelagic or nonpelagic).

	Including stingrays		Excluding stingrays	
Variable	Pelagic	Nonpelagic	Pelagic	Nonpelagic
Number of positive animals	31	21	31	14
Number of negative animals	49	94	49	87
% positive within metabolic group	38.7%	18.3%	38.7%	13.9%

Other sources of contaminants must also be considered during the sampling and culturing process. In several human medicine studies technique is considered a large component of contamination, especially with inexperienced phlebotomists that greatly increase false positive results (Weinbaum et al. 1997; Pavlovsky et al. 2006). In our study the same experienced venipuncturist and microbiologist were used to minimize variability and decrease risk of contamination. Another possible source of contamination is via the needle en route to the vessel from the skin as it traverses muscle or fascia. Since it has been established that there are normal flora in the skin and muscle of elasmobranchs, this should be considered a possible source of contamination in the final assessment of our results (Grimes et al. 1985; Knight et al. 1988). In general, bacterial recovery from blood is low because the number of organisms in the bloodstream of

a clinically abnormal animal or human is usually only at 10 bacteria/mL (Hirsh et al. 1984; Reimer et al. 1997). As such, to mitigate positive results from contamination, Reimer et al. (1997) recommended collecting 20–30 mL of blood per venipuncture . Given that, it is unlikely that the passing of a needle through muscle is a large source that recurrently provides a reserve of bacteria for contamination. To verify this in elasmobranchs, a study must be conducted where a needle is passed through the skin and into the muscle or fascia, but does not penetrate the bloodstream. This should also be performed in comparison with a full venipuncture collection sample in the same animal at the same time point. Similar studies have been conducted in human medicine where a needle was passed through aseptically prepared skin (Zierdt 1983). In that study, recovery rates of skin contaminants due to skin plugs were less than 2%. Thus, while some blood cultures in this study may have contaminants, the percentage is probably low, although additional examination is certainly warranted.

Bacteremias are classified as transient, intermittent, or continuous (Nostrandt 1990). Transient bacteremias indicate an iatrogenic source, such as from a dental procedure or a catheter. Intermittent (or "spontaneous") bacteremias occur owing to a focal source, such as moderate gingival disease or an abscess. Continuous (or "persistent") bacteremias occur secondary to conditions, such as endocarditis and discospondylitis. Once a bacteremia results in a physiological response it is classified as a septicemia (Beers and Berkow 1999). In the case of the elasmobranchs in this study the source of the bacteria is unclear, but it is probable that these bacteremias are spontaneous. Some study individuals were sampled twice at intervals several months apart with different microorganisms cultured. A follow-up study should include serial sampling over a short time period (hourly or daily) to evaluate the time frame and flora for these bacteremias and provide a more definitive classification.

Elasmobranch blood cultures were further evaluated by separating animals into basic lifestyle categories. Animals were categorized as pelagic if their physiology employed constant open-water swimming to maintain adequate respiration (forcing water over the gills) and as nonpelagic if it was possible for the fish to rest on the sea bottom and actively pump water over its gills for ventilation. The latter grouping typically has a lower requirement for oxygen and is in greater contact with other biofilms found on any aquatic substrate. The pelagic animals had a higher percentage of positive cultures than the nonpelagic group did. The importance of this observation is unknown. Since there are autochthonous bacteria in elasmobranch tissues, it is probable that these tissues are a source for bacteremia. Another potential source for bacteria in some sharks are the teeth, which continuously turn over, causing gingival breaks. In animals with crushing plates rather than teeth this is less likely. In veterinary literature, microscopic and molecular studies suggest that the bloodstream may also be colonized with specific benign resident microbes. However, evidence of a true normal microbial bloodstream flora needs to be conclusively demonstrated (Walker 2004).

When the nonpelagic fish were further separated into shark and stingray groups, the stingrays showed a remarkably high percentage of positive blood cultures (50%). Two potentially confounding factors must be considered: first, that the total number of specimens in this study is low, and second, that freshwater stingrays (5 of 14 total) are included in this category and the effects of the freshwater environment on elasmobranch microflora are unknown. Buck (1984) showed that saltwater skates also have a normal flora in their muscles. However, the literature is devoid of data on the status of authochtonous organisms in nonpelagic species.

In both the blood and skin cultures, the most commonly isolated organisms were *P. damsela*, *S. epidermidis*, and *V. alginolyticus*, with some variation in the percentages. *Photobacterium damsela* (formerly *Vibrio damsela*) is considered a normal flora in carcharhinid sharks, but it has been associated with disease in a number of marine species, including elasmobranchs (Pedersen et al. 1997). This organism is also a normal inhabitant of the water column in many aquatic systems and in teleost fish. It has an adhesive affinity for fish mucus, which has been determined as the primary source of entry into intact fish skin (Fouz et al. 1998, 2000). Additionally, it is an organism that can survive in the environment for several months (Fouz et al. 2000). With these properties it is not unusual that this organism was found with the frequency we reported.

Staphylococcus epidermidis is an organism commonly reported in biofilms and itself has the ability to promote biofilm formation through the secretion of mucoid polymers (Adam et al. 2002). Microbial biofilms have been well recognized in aquatic systems; in brief, they occur when microorganisms adhere to a submerged surface (living or nonliving) and produce polymers that aid in adhesion to those surfaces and to each other as part of the normal ecosystem. *Staphylococcus epidermidis* is also an organism previously reported to cause sporadic epizootics in farmed fishes (Huang et al. 1999) and is an opportunistic pathogen in human medicine where it forms biofilms on indwelling catheters, prosthetic devices, and plumbing (Christen-

sen et al. 1982; Donlan and Costerton 2002). The source of some biofilm microorganisms has been reported as human skin, tap water, and other sources in the aquatic environment (e.g., animals living in the environment) (Donlan 2001; Donlan et al. 2002). It is because of the abundance of biofilms in the water that it can be assumed that the source of *S. epidermidis* and many of the organisms reported here is biofilms within the aquarium that houses the animals as well as the animals themselves. A second possible source stems from the fact that in the captive situation reported here divers enter the enclosure daily, which contributes microorganisms to the ecosystem. Similarly in the free-ranging situation animals were caught in areas that were either close to shorelines inhabited by humans (e.g., boats, swimmers, and effluents) or in areas where fishers and crab trappers frequent the waters.

Vibrio alginolyticus is a normal microbe in elasmobranchs as well as in water and on water surfaces (Grimes et al. 1985; Buck 1990). It has also been cited as a cause of disease in fish (Balebona et al. 1998; Liu et al. 2004). Like *P. damsela*, it has the ability to adhere to fish mucus (Fouz et al. 2000). When sampled in Florida waters, *V. alginolyticus* was the most common isolate; in this report it was also the predominant organism in tissues of elasmobranchs, followed by *V. parahaemolyticus* and then *P. damsela*.

The microbial genera of the other isolates reported here have been shown in other elasmobranch studies, though some name changes have occurred (Horsley 1977; Buck 1984, 1990; Buck et al. 1984). The only exception is *Pasteurella pneumotropica*. This organism, however, is associated with biofilms along with many of the other organisms documented here (Rowland 2003; Szymanska 2003).

This study has shown that collecting blood culture samples by standard veterinary protocols results in positive cultures in a variety of elasmobranch fishes. Additional research is warranted, including sampling from multiple venipuncture sites, sampling muscle and liver tissues concurrently, increasing the volume of blood sampled, testing for anaerobic organisms, and sampling serially within a set time frame. To make a definitive diagnosis of septicemia in elasmobranchs, it is obvious that the sole use of a single blood culture would not be sufficient, though it appears to be so in teleosts. While blood hematological profiles may be a useful ancillary diagnostic tool, there are currently very few normal ranges established for elasmobranchs and the circulating cellular response to bacterial agents in this group is poorly understood (Stoskopf 2000; Arnold 2005). The clinician must integrate and interpret clinical presentation, replicated microbial cultures, additional diagnostic procedures, and possibly responses to empirical treatment in order to confirm septicemia in the living elasmobranch.

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