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Isolation of Fecal Coliform Bacteria from the Diamondback Terrapin (*Malaclemys terrapin centrata*)

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Total and fecal coliform bacteria were isolated from the cloaca and feces of the estuarine diamondback terrapin. The majority of samples contained fecal coliforms. *Escherichia coli* was the predominant fecal coliform species isolated, and members of the genus *Salmonella* were isolated from 2 of 39 terrapins. Fecal coliform numbers are used to regulate shellfish harvests, and diamondback terrapins inhabit the brackish-water habitats where oyster beds are found; therefore, these findings have implications for the efficacy of current regulatory parameters in shellfishing waters.

Total and fecal coliform bacteria levels have been used to regulate water usage for decades. In water quality testing, both groups are identified by their ability to ferment lactose, and selective media are used to inhibit the growth of gram-positive organisms. The total coliform group includes many bacteria of nonfecal origin; therefore, the fecal coliform group, which is differentiated from the total coliform group only by its ability to grow at elevated temperatures (44.5°C for shellfish and water samples) (1, 2), has become the most important microbial indicator of water quality.

Elevated fecal coliform counts are commonly the reason that shellfish beds are closed or downgraded in status, particularly in Florida. Several major drawbacks have been observed with respect to the use of fecal coliform indicators in the marine or estuarine waters required by oysters. Survival of fecal coliforms is inversely correlated with salinity (7). Data collected during several studies showed that fecal coliform levels were not correlated with levels of enteric viruses in marine waters (6, 8), and viruses are a major cause of shellfish-associated illnesses (12). *Escherichia coli* is eliminated more rapidly than poliovirus and coliphage from shellfish during depuration (11), so the absence of fecal coliforms may give a false indication of the safety of shellfish.

Implicit in the use of fecal coliform bacteria as indicator organisms has been the understanding that the primary source of these bacteria in natural waters is the feces of warm-blooded animals. In particular, *E. coli* has been regarded as unique to the gastrointestinal tract of warm-blooded animals (2). It has, however, been shown that coliforms and fecal coliforms inhabit the gastrointestinal tracts of some cold-blooded vertebrates. Members of several coliform genera, including *Citrobacter*, *Enterobacter*, and *Klebsiella*, have been isolated from the freshwater turtle *Pseudemys scripta elegans* (9). A survey of the bacterial flora of the gastrointestinal tracts of seven species of freshwater turtles identified *Citrobacter freundii* and *Escherichia coli* (10).

Little is known about the contributions of the feces of cold-blooded (poikilothermic) animals to fecal coliform numbers in estuarine waters. This is an important concern in locations such as northeastern Florida, where oyster harvests have historically had significant economic value and where oyster beds are closed or restricted for harvesting every year in response to elevated levels of fecal coliforms. In many cases, these oyster beds border relatively remote marsh areas with no obvious source of human or high-risk fecal contamination such as feces from cattle and swine. If the human health risk associated with consumption of oysters harvested from such areas is to be assessed meaningfully, it is necessary to determine the animal hosts of the indicator bacteria present in these waters and the potential pathogens associated with them.

This communication reports the isolation of coliform and fecal coliform bacteria from a subspecies of diamondback terrapin, *Malaclemys terrapin centrata*, in estuarine waters of northeastern Florida. This terrapin is limited to brackish water, and its range extends from Cape Cod, Mass., to Corpus Christi, Tex. (5). The brackish-water habitat of the diamondback terrapin is unique, as other aquatic turtles inhabit freshwater or seawater environments.

Sampling sites. Thirty-nine terrapins were captured on a nesting beach in Duval County, Fla., or were trapped in neighboring waters in the spring of 1997 ($n = 29$) and 1998 ($n = 10$) (Table 1). Because the majority of terrapins were caught on a nesting beach (82.1%), females dominated the sample (87.2%). In 1997, only the cloaca (a shared gastrointestinal-urinary-reproductive tract orifice) was sampled for total and fecal coliforms, while in 1998, both cloacal and fecal data were collected.

Sampling techniques. Samples were obtained from the cloaca by swabbing with a sterile cotton-tipped applicator that had been moistened in sterile M-9 buffer (Na_2HPO_4, 7 g liter^{-1}; KH_2PO_4, 3 g liter^{-1}; NH_4Cl, 1 g liter^{-1}; NaCl, 5 g liter^{-1} [pH 7.2]). Applicators were snapped off below the handling point and dropped into a test tube containing 10 ml of M-9 buffer. Samples were immediately transferred to an ice-filled cooler and analyzed the same day.

Because the terrapins defecate only in water, fecal material was obtained by confining terrapins overnight in a bucket containing 2 liters of 25 mM phosphate buffer (pH 7.2) at approximately 28°C. The turtles were rinsed with sterile water before being placed in the buckets. The feces-contaminated buffer was collected aseptically early the following day and was analyzed within 6 h. The minimum sample volume was 50 ml. All turtles were released immediately after the samples were collected.

Water samples were obtained from the confluence of Sister's Creek and Fort George River (an oyster bed site) and from the waters just off the nesting beach. Sister's Creek/Fort George River was sampled on a quarterly basis from February 1997 to

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TABLE 1. Percentage of terrapins testing positive for total and fecal coliforms

Sample method	*n*	% of terrapins positive for:	
		Total coliforms	Fecal coliforms
Cloacal swab	39	66.7	51.2
Fecal collection	10	100.0	80.0

May 1998. The area off the nesting beach was sampled in May 1998 on 8 successive days. Samples were collected in Whirl-pak bags at a depth of 0.2 m. Total and fecal coliform counts per 100 ml were measured by standard techniques (2), i.e., by membrane filtration at Sister's Creek/Fort George River and by multiple tube fermentation off the nesting beach.

Isolation and enumeration of bacteria. Coliform and fecal coliform bacteria were isolated from turtles by multiple tube fermentation and enumerated by most-probable-number (MPN) estimates by using standard techniques (2). Bacterial numbers are expressed as MPN per swab for cloacal samples and as MPN per turtle per night (ca. 15 h) for fecal samples.

Fecal coliform isolates were further characterized by streaking from positive EC broth tubes to eosin-methylene blue agar. All distinct colony types were transferred from eosin-methylene blue to Trypticase soy agar (TSA) plates. Isolated colonies from TSA plates were Gram stained, and the oxidase test was carried out. All colonies tested were gram-negative, oxidase-negative rods which were identified to the species level by the API 20E biochemical test profile (BioMerieux). API strips were processed in accordance with the manufacturer's directions and incubated at 35°C.

Isolates identified as *E. coli* were cultured in the presence of 4-methylumbelliferyl-β-D-glucuronide (MUG) to determine β-glucuronidase activity. The EC-MUG method of *Standard Methods for the Examination of Water and Wastewater* (2) was used.

Total and fecal coliforms were isolated from the cloaca and feces of the majority of terrapins (Table 1). In positive samples, the total coliform MPN per cloacal swab ranged from 4.5 to 1,610, while that of fecal coliforms ranged from 4.5 to 918. Total coliform numbers in feces ranged from 7.3×10^2 to 1.1×10^7 MPN/turtle; fecal coliform numbers in feces ranged from 7.3×10^2 to 1.6×10^6 MPN/turtle (Table 2). Since only the cloaca was sampled in 1997, the data from that sampling are not shown.

The following enteric bacteria were identified from fecal and swab samples that were positive for fecal coliforms: *Citrobacter freundii*, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and a *Salmonella* species. *E. coli* was the most frequently isolated fecal coliform (40.9%), followed by *C. freundii* (22.7%), *K. pneumoniae* (18.2%), and *E. cloacae* (13.6%). One isolate could not be identified by the API 20E system (4.6%). All *E. coli* isolates showed β-glucuronidase activity (MUG^+). Samples from two different turtles (5.1%) yielded *Salmonella* species that were capable of growth (or survival) at 44.5°C.

TABLE 2. Total and fecal coliforms from individual terrapins sampled in 1998

Turtle no.	Total coliforms		Fecal coliforms	
	Swab[a]	Feces[b]	Swab	Feces
322	918	$>3.2 \times 10^5$	918	$>3.2 \times 10^5$
8	4.5	$>3.2 \times 10^5$	4.5	3.2×10^5
79	7.8	$>3.2 \times 10^5$	0	0
214	0	7.3×10^2	0	7.3×10^2
85	348	8.5×10^4	0	9.9×10^3
325	1,610	7.0×10^6	130	4.0×10^4
326	175	1.1×10^7	38.6	1.6×10^6
327	240	3.4×10^6	19.9	8.9×10^4

[a] Coliforms from cloacal swabs are expressed as MPN per swab.
[b] Coliforms from feces are expressed as MPN per turtle per night.

TABLE 3. Total and fecal coliform numbers in natural water samples

Site	Total coliforms/ 100 ml		Fecal coliforms/ 100 ml	
	Geomean	Range	Geomean	Range
Sister's Creek/Fort George River	35.3	18–100	10.7	<3–67
Off nesting beach	25.3	<18–9,180	9.4	<18–44.7

Water samples collected quarterly from the confluence of Sister's Creek and Fort George River satisfied the State of Florida and National Shellfish Sanitation Program requirements for the geometric means of total and fecal coliforms (<70 and <14 per 100 ml, respectively). Water samples collected off the nesting beach were also within the standards (Table 3). This area is, however, currently closed to shellfishing due to historically high levels of fecal coliforms.

Although diamondback terrapins are a protected species, they are not rare in Florida estuaries. Diamondback terrapin densities of 178.3 per acre in Florida's Indian River and 131.1 per acre in the Banana River have been estimated (13). There are no accurate estimates of diamondback terrapin populations in northeastern Florida, but over a 3-year period, a total of 335 female diamondback terrapins were captured on the nesting beach (3a). This fact suggests that the presence of the terrapins may have a significant impact on water quality, especially in locations like nesting beaches, where they are concentrated in the spring.

The current water quality testing paradigm is based on the premise that fecal coliforms and *E. coli* are the inhabitants of the gastrointestinal tracts of warm-blooded animals. Cold-blooded vertebrates are rarely, if ever, considered a potential source of these bacteria in natural waters, in spite of the fact that they are common inhabitants of estuarine systems. The bacteriological data demonstrate that fecal indicator bacteria are readily isolated from the gastrointestinal tract of terrapins that cohabit the brackish-water environments of oysters. Coliforms are present at lower concentrations than one would expect to find in the feces of warm-blooded animals, but the fecal material of the diamondback terrapin is not a concentrated mass, and the shells of the small invertebrates consumed by the terrapins are a major component of their feces.

The survival time of the terrapin fecal coliform isolates in estuarine waters was not measured, but it has been demonstrated that the survival time of both pathogenic and nonpathogenic *E. coli* in natural waters is dependent upon numerous biotic and abiotic factors, including the presence of predators and competitors, temperature, and salinity (3, 7, 14). After a 3-year incubation period, culturable *E. coli* were recovered from artificial seawater microcosms (4), suggesting that long survival times for fecal coliforms are possible when they are excreted into marine environments.

Because it is somewhat difficult to obtain fecal samples from aquatic vertebrates, the limited body of literature that exists regarding the association of fecal coliforms with aquatic turtles has relied largely on the method of cloacal swabs for sampling.

There are obvious disadvantages to this method: since the cloaca is an orifice that receives feces, urine, and eggs, a great deal of variability is introduced into this type of sampling by the recent activities of an individual. In this study, recent oviposition by an individual turtle was not unlikely due to the fact that the majority of terrapins were females captured on a nesting beach. No fecal coliforms were detected on some swabs despite the fact that the feces contained significant numbers of the bacteria; this discrepancy is probably due to recent urination or oviposition.

The fact that the cold-blooded diamondback terrapin sheds coliform bacteria, including *E. coli*, in its feces could be regarded as another note in "exceptions to the rules" of microbiology, but this observation is significant in light of the estuarine habitat that the terrapin shares with oysters. Little is known about the human health risk associated with the normal flora of the diamondback terrapin, but it is unlikely to approach that associated with human fecal contamination. The identification of *Salmonella* species associated with the terrapin gastrointestinal tract is, however, a potential public health concern and should be further investigated.

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REFERENCES

1. **American Public Health Association.** 1970. Recommended procedures for the examination of seawater and shellfish, 4th ed. American Public Health Association, Washington, D.C.
2. **American Public Health Association.** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Bogosian, G., L. E. Sammons, P. J. L. Morris, J. P. O'Neil, M. A. Heitkamp, and D. B. Weber.** Death of the *Escherichia coli* K-12 strain W3110 in soil and water. Appl. Environ. Microbiol. **62:**4114–4120.

3a. **Butler, J.** Unpublished data.

4. **Byrd, J. J., and R. R. Colwell.** 1993. Long-term survival and plasmid maintenance of *Escherichia coli* in marine microcosms. FEMS Microb. Ecol. **12:** 9–14.
5. **Ernst, C. H., J. E. Lovich, and R. W. Barbour.** 1994. Turtles of the United States and Canada, p. 429–441. Smithsonian Institution Press, Washington, D.C.
6. **Gerba, C. P., S. M. Goyal, R. L. LaBelle, I. Cech, and G. F. Bodgan.** 1979. Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. Am. J. Public Health **69:**1116–1119.
7. **Goyal, S. M., C. P. Gerba, and J. L. Melnick.** 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Appl. Environ. Microbiol. **34:**139–149.
8. **Goyal, S. M., C. P. Gerba, and J. L. Melnick.** 1979. Human enteroviruses in oysters and their overlying waters. Appl. Environ. Microbiol. **37:**572–581.
9. **McCoy, R. H., and R. J. Seidler.** 1973. Potential pathogens in the environment: isolation, enumeration, and identification of seven genera of intestinal bacteria associated with small green pet turtles. Appl. Microbiol. **25:**534–538.
10. **Mitchell, J. C., and B. V. McAvoy.** 1990. Enteric bacteria in populations of freshwater turtles in Virginia. Va. J. Sci. **41:**233–242.
11. **Power, U. F., and J. K. Collins.** 1989. Differential depuration of poliovirus, *Escherichia coli*, and a coliphage by the common mussel, *Mytilus edulis*. Appl. Environ. Microbiol. **55:**1386–1390.
12. **Richards, G. P.** 1987. Shellfish-associated enteric virus illness in the United States, 1934–1984. Estuaries **10:**84–85.
13. **Seigel, R. A.** 1984. Parameters of two populations of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida, p. 77–87. *In* R. Seigel, I. Hunt, J. L. Knight, L. Maralet, and N. I. Zuschlag (ed.), Vertebrate ecology and systematics: a tribute to Henry S. Fitch. Museum of Natural History of the University of Kansas special publication no. 10. University of Kansas, Lawrence.
14. **Wang, G., and M. P. Doyle.** 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J. Food Prot. **61:**662–667.