Be careful when you fish: presence of *Vibrio vulnificus* in bait shrimp and fish from the Gulf of Mexico

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Introduction

Vibrio vulnificus is an indigenous member of the estuarine and coastal environments of the Gulf of Mexico. It is considered one of the most dangerous waterborne bacterial pathogens with a fatality rate that can reach 50% for primary septicemia cases. The majority of reported *V. vulnificus* infections are associated with the consumption of raw oysters from the Gulf of Mexico. In addition, *V. vulnificus* can also cause severe wound infections that can be life threatening. Wound infections caused by this bacterium are considered occupational in nature, as they tend to affect fishermen and personnel working at aquaculture facilities. Recently, it has been suggested that global warming and the consequent raise in water temperature could lead to an increase in the number of *V. vulnificus* infections worldwide. Many studies have investigated the natural occurrence of *V. vulnificus* in seafood (mainly in oysters), water and sediments from several marine basins as well as from farm-raised fish. However, very little attention has been paid to the presence of this pathogen on wild fish. DePaola et al. (1994) showed a correlation between densities of *V. vulnificus* in fish and feeding habits, with bottom feeders presenting higher numbers of the pathogen but the authors only examined bacteria associated with the gastrointestinal track. Piercing injuries by fins and spikes are common in sport-fishing activities, creating an opening for *V. vulnificus* to penetrate into the anglers' skin with the associated risk for infection. The Gulf of Mexico supports a large industry of recreational fisheries. Data from NOAA show that marine recreational participants took more than 24.1 million trips catching 190 million fish from the Gulf of Mexico and surrounding waters in 2008. In the Gulf, recreational species include speckled trout, redfish, snapper, grouper, cobia, jacks, and tuna among others. Many of these fishes possess spiny fin rays in their dorsal fin, which act as needles and may cause puncture wounds in anglers. The objective of the presented study was to investigate the prevalence of *V. vulnificus* associated with sport fishing species and bait shrimp in the Gulf.

Materials and Methods

Sampling efforts started in November 2009 and have continued throughout 2010. Fish were caught using rod and reel and casting net at various locations on the Gulf. Sampling locations included Dauphin Island, AL and Ocean Springs, MS. Dorsal fin clips from captured fish were aseptically removed and placed into Alkaline Peptone Water (APW). Samples were

allowed to incubate overnight at room temperature. Upon arrival to the lab, 100 µl of APW was plated onto mCPC and TCBS plates and incubated for 18-24h at 30°C. Colonies displaying the typical *V. vulnificus* morphology were randomly selected, purified on Marine Agar and stored as glycerol stocks for further testing. Bacterial DNA was extracted using standard protocols and used as template for PCR. Putative isolates were identified as *V. vulnificus* using the specific PCR method described by Campbell and Wright (2003).

Bait shrimp purchased at different local bait shops at Mobile (MB), Dauphin Island (DI), and Ocean Spring (OS) were sampled for *V. vulnificus* enumeration using FDA-approved method (Note: no commercial shrimp samples were found in July due to the oil spill). Randomly selected putative *V. vulnificus* isolates on mCPC were collected, confirmed as described above and added to our collection for further characterization. All *V. vulnificus* isolates, from fish and shrimp, were characterized according to Nilsson et al. (2003) and Arias et al. (1997).

Results and Conclusions

Table 1 summarizes the result obtained from bait shrimp. As was expected due to low water temperatures in winter, samples obtained in November and March yielded non-detectable *V. vulnificus* numbers. However, June, July and August samples showed numbers of *V. vulnificus* higher than 10^5 MPN/g.

Table 1. Densities of *Vibrio vulnificus* in bait shrimp with time

Date collected (mm/dd/yyyy)	*Vibrio vulnificus densities* (MPN·g^{-1})		
	MB, AL	DI, AL	OS, MS
11/22/2009	<3	ND[a]	ND
03/26/2010	<3	<3	<3
06/02/2010	ND	>1.1X10^5	>1.1X10^5
07/18/2010[b]	ND	ND	>1.1X10^5
08/19/2010[c]	ND	>1.1X10^4 >1.1X10^5	ND

[a] ND, not determined

[b] shrimp were collected from a marsh area by the authors using a cast net

[c] two different shrimp samples were collected at two sites in DI

Regarding fish samples, a total of 140 fish have been sampled to date with 84 yielding positive *V. vulnificus* colonies (60%). Table 2 displays water temperature and salinity and ratio of Vv-positive fish of each sampling. The increase in prevalence correlates well with an increase in water temperature and favorable salinities. Table 3 shows number of Vv-positive percentage per fish species. Our data show that handling bait shrimp and finfish from the Gulf of Mexico constitute an inherit risks of exposure to *V. vulnificus* even during the colder months.

Table 2. Temporal distribution of fish examined for presence of *V. vulnificus*

Date (mm/dd/yy)	Salinity (ppt)	Water Temp. (°C)	No. of total fish	No. of Vv+ fish	Vv pos.(%)
11/17/09	18	16	19	10	53%
03/26/10	14	17	30	16	53%
06/02/10	14	29	20	12	60%
06/16/10	10	31	25	20	80%
07/18/10	8	30	23	19	83%
08/18/10	24	30	23	7	30%
Total			140	84	60%

Table 3. Number of positive fish for *V. vulnificus* distributed by species

Fish name (Latin name)	No. of total fish	No. of Vv + fish	Percentage
Black drum (*Pogonias cromis*)	1	0	0%
Croaker (*Micropogonias undulatus*)	26	19	73%
Hardhead catfish (*Ariopsis felis*)	5	5	100%
Ladyfish (*Elops saurus*)	4	2	50%
Mullet (*Mugil cephalus*)	9	4	44%
Pigfish (*Orthospristis chrysotera*)	3	1	33%
Pinfish (*Lagodon rhomboides*)	7	5	71%
Pogie (*Brevoortia patronus*)	1	0	0%
Red drum (*Sciaenops ocellatus*)	4	4	100%
Sand seatrout (*Cynoscion arenarius*)	17	10	59%
Sheepshead (*Archosargus probatocephalus*)	1	1	100%
Silver perch (*Bairdiella chrysoura*)	23	10	43%
Southern flounder (*Paralichthys lethostigma*)	14	10	71%
Southern Kingfish (*Menticirrhus americanus*)	5	2	40%
Spadefish (*Chaetodipterus faber*)	2	2	100%
Spanish mackerel (*Scomberomorous aculatus*)	1	0	0%
Speckled trout (*Cynoscion nebulosus*)	15	8	53%
Sting ray (*Dasyatis sabina*)	1	1	100%
Toadfish (*Opsanus beta*)	1	0	0%

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