

Occurrence of Fecal Coliforms in Surface Waters in the Tiger Point Golf Course  
Drainage Area, Santa Rosa County, FL.

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## Occurrence of Fecal Coliforms in Surface Waters in the Tiger Point Golf Course Drainage Area, Santa Rosa County, FL.

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### Summary

From 7/16/96 to 12/17/96 fecal coliforms were enumerated in water and oyster tissue samples obtained from the Tiger Point Golf Course area. Unsanitary conditions (fecal coliform counts exceeding a standard of 200 per 100 ml) were found in water samples obtained from Boones Bayou and a ditch which drained into Boones Bayou. Low fecal coliform numbers (sanitary conditions) were observed in water samples collected from storage ponds receiving chlorinated effluent from the South Santa Rosa Utility Company's wastewater treatment facility and from surface waters on the golf course. Oysters (possessing < 20 fecal coliforms per 100 ml) placed 90 meters from the mouth of Boones Bayou, accumulated and concentrated *Escherichia coli* and other fecal coliforms.

### Introduction

A municipal waste water treatment facility (WWTF) located on Tiger Point Golf Course in Santa Rosa County, FL. utilizes its effluent in spray irrigation. The Tiger Point WWTF stores treated, chlorinated effluent in holding ponds. From these ponds the effluent is sprayed onto the golf course for irrigation. The ecological impact of spray

irrigation on Santa Rosa Sound was assessed during 1996. One indicator of impact was contamination by fecal coliforms which is the subject of this report.

Fecal coliforms were monitored in this study because they are the recommended bacterial indicators for measuring the sanitary quality of recreational waters and shellfish in the United States. Fecal coliforms are thermotolerant members of the total coliform group. They are defined as gram-negative nonspore-forming rods that ferment lactose in  $24 \pm 2$  hr at  $44.5 \pm 0.2^{\circ}$  C with the production of gas in a multiple-tube procedure or produce acidity with blue colonies in a membrane filter procedure. In 1968 a National Technical Advisory Committee set the current water quality safety standard of 200 fecal coliforms per 100 ml of water (1). For commercial oysters the retail limit for fecal coliforms is 230 per 100 g. To determine if the above criteria were exceeded, surface water samples and oysters, which accumulate and concentrate bacteria, were analyzed for fecal coliforms to assess sanitary quality.

## **Materials and Methods**

Duplicate water samples, collected from 7/16/96 to 10/11/96, were taken at a depth of 0.1 meter, stored on ice, and analyzed in the laboratory within 6 hr of collection. Location, time, and hydrological parameters were recorded for each sample. Dissolved oxygen (mg/L), temperature ( $^{\circ}$ C), pH and salinity (parts per thousand, ‰)

were measured with a Hydrolab Scout II<sup>1</sup>.

During September and December oysters, free of fecal coliforms, were caged at selected locations for 12 and 21 days, respectively. Exposed oysters, 5 per location, were analyzed for fecal coliforms. The direct membrane filter method (MF) and the most probable number (MPN) method (2) were used to enumerate fecal coliform bacteria in surface water samples. In reporting fecal coliform counts using the direct membrane filter method, a percent verification correction factor was used. For enumeration of fecal coliforms in oyster tissue, chlorinated effluent and turbid water samples, a multiple tube fermentation, 5-tube MPN assay (3) was used. *Escherichia coli*, a member of the fecal coliform group, was also enumerated using the MPN method by transferring inocula from lactose broth tubes that showed gas or growth to EC broth with 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG) and incubating at 44.5<sup>o</sup> C for 24 hr. All EC tubes showing gas were scored positive for fecal coliforms and all EC tubes showing fluorescence under long wave UV-illumination were scored as positive for *E. coli*.

## Results and Discussion

Sampling stations are briefly described in Table 1. Sampling dates, temperature, salinity, dissolved oxygen, and pH of the water are also shown. Water temperatures

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<sup>1</sup>Mention of trade names of commercial products does not imply endorsement by the U.S. Environmental Protection Agency

ranged from 26 to 32° C. A maximum pH of 9.7 was measured at the WWTF emergency holding pond, Station 2. A minimum pH of 6.1 was measured at Boones Bayou, Station 3. Salinities ranged from freshwater in the wastewater holding pond, golf course ponds and drainage ditches of Stations 1, 2, 14, 16 and 19 to brackish water (20 - 25 ‰) in water samples taken from Santa Rosa Sound. Low dissolved oxygen concentrations (< 1.0 mg/L) were measured in the drainage ditches (Stations 0 and 19) and in Boones Bayou.

Fecal coliform enumerations using both MF and MPN procedures are reported in Table 2. To assure accuracy with our MF enumeration procedure, replicate samples were analyzed by personnel at the Florida Department of Environmental Protection (DEP). Replicate samples from Stations 3 and 19 were taken and analyzed on 8/12/96. DEP reported a fecal coliform count of 3,500 and 3,380 per 100 ml from Stations 3 and 19, respectively. We obtained lower counts of 1,130 and 1,700 fecal coliforms per 100 ml from Stations 3 and 19, respectively. A possible explanation for this difference is that our verification procedure differed from the method used by the DEP. We calculated a percent verification and applied this figure as a correction factor to our direct test results (2). This correction factor was not used by DEP. Instead, random and atypical looking colonies were verified. Thus, counts we obtained (before correction) of 2,260 and 3,050 fecal coliforms per 100 ml for Stations 3 and 19, respectively, were in agreement with the results reported by the DEP.

To further validate our data of 8/12/96, we enumerated fecal coliforms from Station 3 using the MPN procedure. The MPN procedure is recommended as a quality control

measure to be run in parallel with the MF method whenever a highly turbid sample is encountered or when the coliforms may be injured (eg., chlorinated wastewater or high salinities). The MPN technique yielded a fecal coliform count (95% CL) of 7,000 (2,300-17,000) per 100 ml water (Table 2). This count (approximately 6X higher than the count obtained by the MF method) suggests that the coliforms were injured in this water sample, possibly due to the high salinity (21‰)(Table 1).

Water samples taken from the storage ponds receiving the chlorinated effluent (Stations 1 and 2) yielded fecal coliform levels below the State standard of 200 fecal coliforms per 100 ml. These results were confirmed with the MPN procedure to assure that any chlorine-injured coliforms were being enumerated (Table 2). These data show that the WWTF was discharging a final chlorinated effluent which meets the fecal coliform standards for surface waters. This water is used to irrigate the golf course and, samples taken from surface waters on the golf course also contained low numbers of fecal coliforms, verifying the effectiveness of the chlorination.

This study revealed fecal coliform contamination in a residential section near the western boundary of the golf course. Water samples from Stations 3 and 19 taken on 8/12/96 contained fecal coliforms in numbers that greatly exceeded the water quality safety standard. Station 19 is a drainage ditch that flows between homes south of the WWTF. This ditch empties into Boones Bayou, Station 3, a heavily urbanized area.

The extent of fecal coliform contamination in Santa Rosa Sound adjacent to Boones Bayou was also studied. Because fecal coliforms are inhibited in saltwater, we chose oysters as biomonitors of fecal coliform contamination. Oysters are filter feeders

which readily accumulate coliforms and other bacteria from their environment.

Therefore, these bivalves were used at different locations during September and December in Santa Rosa Sound to determine the extent of the fecal contamination.

Results of these experiments are presented in Table 3.

Oysters possessed (< 20) fecal coliforms at the start of both the September and December experiments (Table 3). In the September experiment, oysters caged at the mouth of Boones Bayou (Station 3, 15 meters from the drainage ditch) became heavily contaminated with fecal coliforms (13,000 per 100 g) after 12 days of exposure. Importantly, these fecal coliforms were shown to be *Escherichia coli*, which strongly suggests that their source was from feces of warm-blooded animals. Heavy contamination of these oysters was not surprising since fecal coliform counts of water taken from this site (499 fecal coliforms per 100 ml of water) were higher than the standard set by the State of Florida (Table 3). Fecal coliform numbers were below detection limits in water samples at Stations 4 and 5. However, after 12 days the transplanted oysters had accumulated 230 fecal coliforms per 100 g (Station 4) and 170 fecal coliforms per 100 g (Station 5). Also of importance was the fact that 74% and 41% of the fecal coliforms present in Station 4 and 5 oysters, respectively, were *E. coli*. In approximately 3 days oysters can purge themselves of fecal coliforms when placed into uncontaminated water. Thus, these oysters were probably exposed to a continuous or repetitive input of fecal coliforms during the 12- day period in the September experiment.

Results from the December experiment support the conclusions from the

September experiment. Fecal coliforms were below detection limits in water sampled from Stations 4, 9 and 12. However, oysters exposed at Stations 4 and 9 accumulated and concentrated fecal coliforms from the water (Table 3). Station 4 is approximately 90 meters from the mouth of Boones Bayou. Oysters exposed at Station 12, a relatively pristine site approximately 4 kilometers east of Boones Bayou, were not contaminated with fecal coliforms. Oysters exposed at Station 9 contained 790 fecal coliforms per 100g (Table 3). This result was unexpected. Station 9 is, however, at the mouth of a small bayou. We have not done extensive sampling in this area and further testing may be warranted. *E. coli* was not found in oysters from the December experiment. Perhaps this coliform is not as cold-tolerant as other members of this group and died before entering Santa Rosa Sound.

In conclusion, the data indicate that during this study the practice of spraying chlorinated effluent onto the golf course and the overflow drainage did not result in a fecal coliform contamination problem in the area. A beneficial supplemental study would be to corroborate these findings after a heavy rain event. In contrast to the above, high coliform counts in surface water samples were detected just southwest of the golf course, and caged oysters at the mouth of a small bayou in this area became heavily contaminated with fecal coliforms after 12 days of exposure.

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## References

1. National Technical Advisory Committee. Water Quality Criteria . Federal Water Poll. Control Admin., Dept. of the Interior, Washington, DC. 1968.
2. Microbiological Methods for Monitoring the Environment - Water and Wastes. R. Bordner and J. Winter (eds.) U.S. Environmental Protection Agency EPA-600/8-78-017, 1978.
3. American Public Health Association (APHA). 1985. "Standard Methods for the Examination of Water and Wastewater." 16th Edition. Amer. Publ. Health Assoc., Washington, DC.

Table 1. Sampling stations and properties of the water samples. T : temperature, °C; S : salinity, parts per thousand; DO : dissolved oxygen, mg/L

Station	Description	Sampling Dates																	
		7/16/96				7/22/96				8/12/96				9/18/96				10/11/96	
		T	S	DO	pH	T	S	DO	pH	T	S	DO	pH	T	S	DO	pH	T	S
0	Drainage inlet (Bay St.)	27	20	0.5	6.7														
1	WWTF (Pond for treated effluent)	31	0.3	6.4	7.2					30	0.3	5.8	7.0						
2	WWTF (Emergency holding pond)					32	0.1	6.9	9.2					28	0.1	11	9.7	19	0.1
3	Boones Bayou	29	18	2.9	6.9	30	8.3	1.1	6.8	30	21	0.8	6.8	26	9	1.5	6.1	18	22
4	Sound (near channel to Boones Bayou)													28	23	5.7	7.4		
5	Sound (Residential dock)													28	22	5.5	7.7		
6	Residential Finger Canal (SE of GC)													29	21	4.8	7.4		

Table 1. Cont.

Station	Description	Sampling Dates																	
		7/16/96				7/22/96				8/12/96				9/18/96				10/11/96	
		T	S	DO	pH	T	S	DO	pH	T	S	DO	pH	T	S	DO	pH	T	S
7	Golf course pond (Waterfront condos)	30	16	4.1	7.1														
13	Culverts from golf course													29	7.0	3.6	6.9		
14	Freshwater pond (E. side of GC)	30	0.1	8.2	7.8														
16	Freshwater pond (E. side of GC)													29	0.3	14	9.0		
18	Sound - undeveloped area (E. side of GC)													28	22	6.3	7.7		
19	drainage ditch - elephant ear					28	0.1	0.9	7.1	27	0.2	2.6	6.9						

Table 2. Fecal coliforms per 100 mls water. MPN : most probable number. Numbers in parentheses are 95% confidence limits. NR : no result, too many colonies on membrane filter at highest dilution (1 ml filtered).

Sampling Station	DATES					
	7/16/96	7/22/96	8/12/96	9/18/96	9/24/96	10/11/96
0	< 10					
1	< 10		< 20 MPN			
2		< 10		117	< 10	40 (10-170) MPN
3	186	270	1,130 7000 (2,300-17,000) MPN	499	300 estimated count	468 790 (250-1900) MPN
4				< 10		
5				< 10		
6				22 estimated count		
7	< 1					
13				37		
14	< 1					
16				45		
18			< 10	< 10		
19		NR	1,700			103

Table 3. Accumulation of fecal coliforms by oysters placed in areas of Santa Rosa Sound receiving drainage from the Tiger Point area. MF: membrane filter; MPN: most probable number (95% CL)

**September Experiment**

Measurements	Beginning of exposure - 9/18/96			End of exposure - 9/30/96		
	Station 3	Station 4	Station 5	Station 3	Station 4	Station 5
fecal coliforms/ 100 ml water	499 MF	<10 MF	<10 MF	Not done	Not done	Not done
fecal coliforms/ 100 g oysters	<20 MPN	<20 MPN	<20 MPN	13,000 (3,500 - 30,000)	230 (70 - 700)	170 (50 - 460)
<i>Escherichia coli</i> /100 g oysters	<20 MPN	<20 MPN	<20 MPN	13,000 (3,500 - 30,000)	230 (70 - 700)	70 (10 - 170)

**December Experiment**

Measurements	Beginning of exposure - 11/26/96			End of exposure - 12/17/96		
	Station 4	Station 9	Station 12	Station 4	Station 9	Station 12
fecal coliforms/ 100 ml water	Not done	Not done	Not done	< 20 MPN	< 20 MPN	< 20 MPN
fecal coliforms/ 100 g oysters	<20 MPN	<20 MPN	<20 MPN	330 (110-930)	790 (250-1900)	20 (<5-70)
<i>Escherichia coli</i> /100 g oysters	<20 MPN	<20 MPN	<20 MPN	< 20 MPN	< 20 MPN	< 20 MPN