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Microbial Impact of Canada Geese (*Branta canadensis*) and Whistling Swans (*Cygnus columbianus columbianus*) on Aquatic Ecosystems

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Quantitative and qualitative analyses of the intestinal bacterial flora of Canada geese and whistling swans were carried out with the finding that wild birds harbor significantly more fecal coliforms than fecal streptococci. The reverse was typical of captive and fasting birds. Neither *Salmonella* spp. nor *Shigella* spp. were isolated from 44 migratory waterfowl that were wintering in the Chesapeake Bay region. Enteropathogenic *Escherichia coli* were detected in seven birds. Geese eliminated 10^7 and swans 10^9 fecal coliforms per day. Results of in situ studies showed that large flocks of waterfowl can cause elevated fecal coliform densities in the water column. From the data obtained in this study, it is possible to predict the microbial impact of migratory waterfowl upon aquatic roosting sites.

Waterfowl have been reported to excrete disproportionately large amounts of fecal indicator bacteria (13) and occasionally to harbor enteric pathogens (11, 15). Exceptionally high concentrations of wastes from duck farms have caused restrictions to be placed on nearby shellfish beds (8) and have been cited as a factor in algal blooms (17). This suggests that dense populations of waterfowl could have a serious impact on aquatic resting and feeding sites. The extent of such impact would depend principally on the numbers of fecal indicator bacteria discharged, the accumulation of organic matter, and the presence or absence of pathogens in the bird feces. The size of the birds and of the bird populations may also be critical, since Brierly and co-workers (4) reported that flocks of waterfowl failed to cause detectable changes in the bacterial populations of a study site located in the Bosque del Apache Preserve.

If the fecal output of individual waterfowl and of migrating flocks of waterfowl can be quantified, the severity of impact on the water quality at a roosting site caused by a given flock could be estimated. Thus, the objectives of the study reported here were to obtain qualitative and quantitative measures of the bacterial flora of *Cygnus columbianus columbianus* (whistling swan) and *Branta canadensis* (Canada goose) and to determine the fate of bacteria released by the birds in the waters of selected aquatic flocking areas of the Chesapeake Bay.

MATERIALS AND METHODS

Estimation of fecal loads derived from waterfowl. Fresh fecal material from wild waterfowl was periodically collected. Additionally, cages with removable bottom trays were constructed to monitor avian fecal output on a routine basis. Birds were confined in the cages for periods of 4 to 24 h. While caged, the birds were fed corn, turkey feed, water, and grit. Fecal material was collected on presterilized aluminum foil placed on the trays located beneath the cage floor. Fecal wet weight was measured, and bacteriological analyses were carried out on each sample.

At the field sample sites (Fig. 1), waterfowl species were identified and counted weekly using procedures established in earlier studies (20).

Sampling methods. Fecal material, collected from the cages at the field sites and from autopsied birds, was used for the quantitative bacteriological analyses. In addition, cloacal swabs were also taken during the field studies. Fresh fecal material was iced for transport to the laboratory. Within 6 h all of the samples were inoculated into bacteriological media for subsequent analyses. Interim bacterial growth or die-off was less than 10% of the initial densities, a result obtained in this study that was consistent with findings reported by Crowther (6).

For 1 year the sample sites shown in Fig. 1 and described in Table 2 were monitored at biweekly intervals when birds were present in the Chesapeake Bay and monthly when they had migrated away from the bay region. Correlation matrixes relating bacterial densities to water temperature and to bird populations were calculated using a biomedical computer program (U.C.L.A.) package on a University of Maryland Univac 1108. Data from a total of 90 samples were ana-

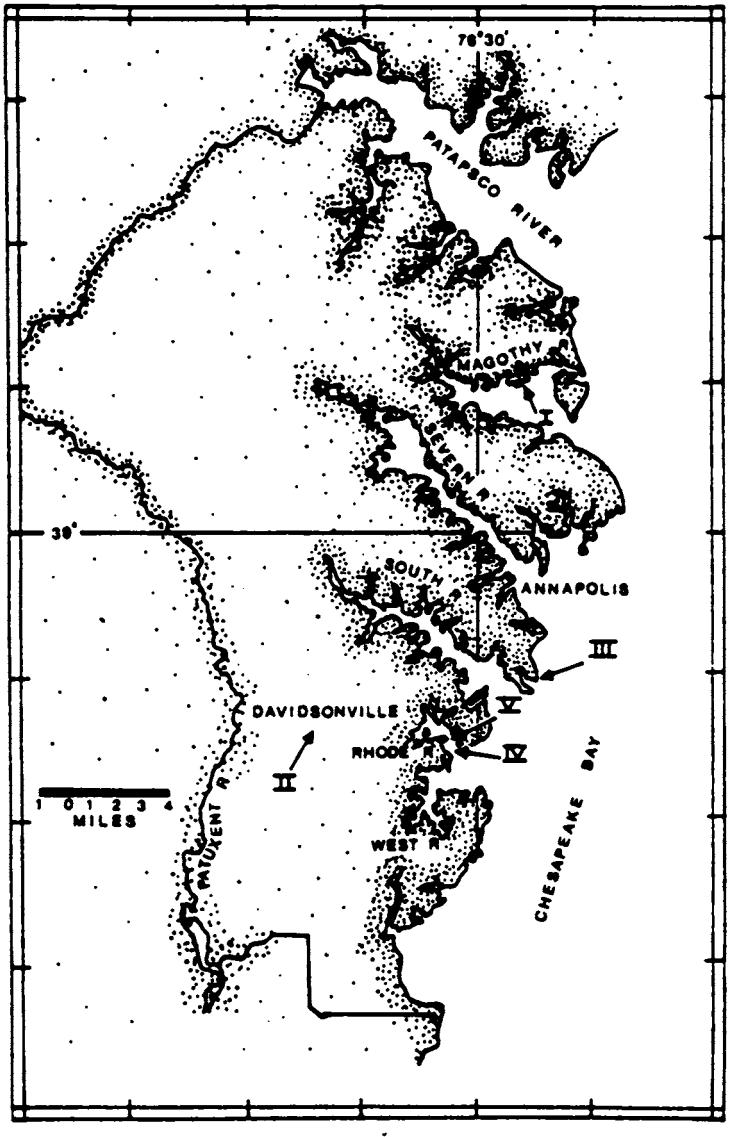

FIG. 1. *Sample stations: Lake Shore Pond (I), Double Gate Road Pond (II), Fishing Creek Cove (III), Canninghouse Cove (IV), and Locust Point (V).*

lyzed, with each of the samples including 10 parameters.

Surface water (upper 5 cm) was collected in sterilized Nalgene bottles (250 ml), and the upper 15 cm of the sediment was collected using a Petite Ponar grab (Wildlife Supply Co., Saginaw, Mich.). Volumes of 20 ml of sediment were immediately diluted in 180 ml of estuarine salt solution (5) and transported in iced containers with the water samples. The water and sediment samples were inoculated into bacteriological media immediately in the field or in the laboratory within 12 h after collection.

Methods employed for enumeration of aerobic heterotrophic bacteria. Fecal samples (1.0 g) or cloacal swabs were diluted in sterile phosphate-buffered saline adjusted to pH 7.2, plated on Trypticase soy agar (BBL), and examined after incubation for 72 h at 37°C.

Water and sediment samples were diluted in chilled estuarine salt solution and plated on ESWYE medium (5). The plates were counted after incubation for 7 days at 20°C.

Enumeration of aerobic sporeforming rods. Fecal samples were diluted 1:10 in phosphate-buffered

saline and were subjected to 80°C for 20 min. The samples were immediately cooled and plated as indicated above for aerobic heterotrophic bacteria, and colony counts were made after incubation for 72 h at 37°C.

Indicator organisms. The most probable numbers of total coliforms, fecal coliforms (FC), and fecal streptococci (FS) were determined following procedures recommended for water analysis (1). A five-tube series of the most-probable-number procedure was used. For presumptive determinations, lactose broth (Difco) was used for coliforms and sodium azide dextrose broth (Difco) was used for FS. Confirmatory tests for total coliforms were done using brilliant green lactose bile broth (Difco) and Levine eosin methylene blue agar (BBL). Confirmation for FC was in EC broth (Difco) inoculated at 44.5°C; for FS ethyl violet azide broth (Difco) was inoculated at 37°C.

Anaerobic heterotrophic bacteria. Freshly collected fecal samples (1.0 g) were diluted immediately after collection into a liquid medium recommended by Holdeman and Moore (14) and then inoculated into thioglycolate agar (2.5% final agar concentration) without dextrose (Difco). In addition, anaerobes were enumerated by the dilution-to-extinction method employing thioglycolate broth without dextrose. Inoculated cultures were transferred to anaerobic jars (BBL, GasPak) immediately upon completion of the dilution and inoculation procedures. Colony counts or recordings of the positive tubes were made after incubation of the media at 37°C for 4 days.

***Clostridium* spp.** The fecal samples were diluted and heat shocked as described above for the aerobic sporeforming bacteria counts. Colonies on plates of thioglycolate agar (2.5%) without dextrose were counted after incubation for 4 days in anaerobic jars held at 37°C.

Enrichment for sporeforming anaerobes was accomplished by heat treatment of the fecal samples, followed by inoculation into cooked-meat medium (Difco) or thioglycolate medium without dextrose (Difco). The inoculated media were incubated anaerobically at 37°C for 10 days before heat treatment. Colonies were isolated on blood agar and on thioglycolate agar (2.5%) without dextrose. Cell morphology and colony characteristics on blood agar, reaction in cooked meat medium, dextrose fermentation, and gelatin liquefaction were features recorded and employed for presumptive identification (10). Strains suspected to be *Clostridium* spp. were tested for toxicity in mice following the procedure of Cruickshank et al. (7). *Clostridium* spp. were identified by using gas-liquid chromatography analysis, which yielded metabolic end-product profiles (14).

***Salmonella* spp.** Procedures employed for detection of *Salmonella* spp. included direct inoculation of selective media, e.g., Levine EMB agar (BBL), SS agar (Difco), XLD agar (BBL), and bismuth sulfite agar (BBL), and selective enrichment using GN (BBL), tetrathionate (BBL), and Selenite F broth (BBL). Strains presumptively identified as *Salmonella* spp. were transferred to TSI agar slants (Difco) for subsequent confirmation using the API-20E system (Analytab). Organisms isolated from feces of 44 waterfowl and from environmental samples were screened for *Salmonella*.

Enterotoxin-producing *Escherichia coli*. Randomly selected *E. coli* isolates were tested for production of heat-labile enterotoxin using a miniculture assay similar to that developed by Sack and Sack (18) and Donta et al. (9). Y1 mouse adrenal cells were maintained in RPMI 1640 (Flow Labs) medium supplemented with 10% (vol/vol) fetal bovine serum, penicillin (10 U/ml), and streptomycin (10 μg/ml) and incubated at 37°C in 5% CO_2. The cells were subcultured into 96-well tissue culture dishes (Costar) at a seeding density of approximately 10^5 cells per well. At this density monolayers were confluent and suitable for use after incubation for 1 day.

Logarithmically growing *E. coli* isolates were harvested from brain heart infusion broth (Difco) and exposed to Y1 mouse adrenal cells. After 5 min the *E. coli* were removed, and the monolayer cells were washed twice with phosphate-buffered saline and provided with fresh tissue culture medium. The Y1 cells were observed for 24 h for typical "rounding." Known positive and negative controls were included in each assay.

RESULTS AND DISCUSSION

The primary objective of the study was to determine whether waterfowl migrating along the Atlantic Flyway and wintering in the tributaries of the Chesapeake Bay adversely affected the water quality at their resting and feeding sites. The presence of potentially pathogenic bacteria and of elevated concentrations of fecal indicator organisms were criteria judged to be indicative of adverse impact. The study reported here covered a full year cycle during 1976–1977.

The common Canada goose and the whistling swan were monitored, since they are among the dominant migratory waterfowl populations of the Chesapeake Bay region (20, 24; W. J. L. Sladen, unpublished data).

Freshly collected fecal matter from caged birds and from autopsied wild swans and geese, comprising a total of 44 samples, did not yield *Salmonella* spp. It can be argued that the sample size, i.e., 0.001% of all the waterfowl in the Chesapeake Bay at the time of sampling, was small. Nevertheless, it can be concluded that only a minority of birds, if any, were carrying *Salmonella* spp. Furthermore, *Salmonella* spp. could not be recovered from the four aquatic roosting sites included in this study. In previous studies, salmonellae have been recovered from some flocks of waterfowl (2, 11, 15, 16) but not from others (3, 19, 23).

Although no *Salmonella* were detected, a random selection of 75 *E. coli* isolates from the various waterfowl yielded 7 that were identified as enterotoxin-producing *E. coli*. The individual waterfowl harboring these enterotoxigenic *E. coli* were all confined to Lake Shore Pond (sample site I, Fig. 1).

Most of the facultatively anaerobic bacteria

TABLE 1. *Bacterial population sizes associated with fecal content of whistling swans and Canada geese*

Waterfowl	No. of bacteria per g of feces ± log SD[a]						FC/FS ratio	Ratio of aerobic/anaerobic TVC	Avg contribution per bird per 24 h		
	TVC	Aerobic spore-forming rods	FC	FS	Anaerobic TVC	Anaerobic spore formers			Wt of feces (g)	FC	FS
Whistling swans											
Wild[b]	3.6×10^7 (±1.19)	2.7×10^3 (±1.36)	2.5×10^6 (±1.45)	6.4×10^5 (±1.05)	3.0×10^8 (±1.88)	1.4×10^3 (±0.44)	3.9	8.3	317	7.9×10^8	2.0×10^8
Fasting[c]	7.3×10^4 (±0.39)	3.7×10^2 (±0.92)	4.8×10^1 (±0.30)	3.4×10^2 (± 0.25)	1.8×10^7 (±0.82)	1.1×10^4 (±1.63)	0.1	246.6	ND[d]	ND	ND
Canada geese											
Wild[b]	7.4×10^6 (±1.04)	ND	3.6×10^4 (±1.91)	3.9×10^3 (±1.92)	6.2×10^8 (±0.56)	8.5×10^3 (±0.65)	9.2	83.7	250[e]	9×10^6	9.8×10^5
Captive[c]	9.6×10^4 (±0.39)	8.4×10^3 (±1.04)	3.6×10^2 ±1.22)	6.2×10^2 (±1.02)	1.8×10^7 (±0.41)	3.3×10^3 (±0.88)	0.6	187.5	202	7.3×10^4	1.2×10^5

[a] Log SD, Log standard deviation. TVC, Total viable count.
[b] Log averages calculated from seven samples.
[c] Log averages calculated from four samples.
[d] ND, No data.
[e] Estimated output.

isolated were *Escherichia* and *Streptococcus* spp. The fecal indicator bacteria associated with waterfowl were quantified on a per-bird basis, as well as in situ contamination. In the former, the results are reported on the basis of grams excreted per bird per day, multiplied by the indicator organisms count per gram excreted (see Table 1).

Assessment of the numbers of bacterial indicator organisms was complicated by the fact that the number of FC and FS varied from bird to bird. Furthermore, it was found that different species of waterfowl and waterfowl of the same species maintained on different diets harbored substantially different total numbers of intestinal bacteria, an observation reported to occur in warm-blooded animals (21, 22) but heretofore not in birds. Therefore, as shown in Table 1, wild swans feeding on aquatic vegetation and insects, fasting swans sampled during extreme cold spells, wild geese feeding on aquatic vegetation, and captive geese feeding primarily on corn were grouped individually. Total viable, aerobic, heterotrophic counts and numbers of fecal indicator organisms were always highest in waterfowl feeding in the wild. Among these, the bacterial counts were similar to those reported for other warm-blooded animals (22).

Results obtained in this study differed from those reported by Geldreich and Kenner (13) in that waterfowl feeding in the wild yielded larger numbers of FC than FS. Thus, the FC/FS ratios resembled those typical of human enteric flora. The data reported by Geldreich and Kenner (13) may have been obtained from artificially fed or fasting birds. In any case, the results reported here suggest that it is not possible to separate avian fecal contamination from human fecal contamination on the basis of shifts in FC/FS ratios.

While the aerobic bacterial counts varied among the different avian populations, depending on mode of feeding, the numbers of anaerobic non-spore formers and spore formers remained relatively constant. *Clostridium perfringens* and toxin-producing *Clostridium botulinum* were routinely isolated from the intestinal tracts of the waterfowl. However, *Clostridium tetani* was not among 150 anaerobic sporeforming species isolated from the samples examined in this study.

FC were found to be useful indicators of avian fecal contamination since they comprised 1 to 10% of the total nonanaerobic bacterial flora of geese and swans feeding in the wild. In a 24-h period, it is estimated that a single swan will eliminate up to 10^9 FC, and a goose will excrete 10^7 FC. The effects of fecal contamination from known concentrations of waterfowl in given bodies of water were determined by examining three experimental and two control sites in the Chesapeake Bay region (see Fig. 1 and Table 2).

One site (positive control) was an artificial pond (Lake Shore) in which approximately 25 captive swans and geese were maintained. At this site and at others, swans and geese excreted repeatedly into the water. "Bird hours" were calculated as the number of birds multiplied by the number of hours spent in the vicinity of the pond. Theoretical waterfowl inputs were determined as described in Table 2. It was calculated that the fecal load from swans and geese in the pond was 3.6×10^9 FC within a 24-h period.

TABLE 2. *FC recovery from aquatic environments*

Sample site					Theoretical waterfowl input on a 24-h basis			FC recovered from 100 ml of water	
Station	Environmental characteristics	Bird population	Salinity (‰)	Calculated water vol (100-ml volumes)[a]	Bird hours[b]	FC[c]	FC/100 ml[d]	Sediment	Surface
Lake Shore Pond	Artificial pond, excavated during the fall of 1975. Location: north of Annapolis, Md.	Captive swans and geese	4–9	7.5×10^5	220 0[e]	3.6×10^9	4.8×10^3	3.5×10^4 4.9×10^2	2.4×10^3 1.0×10^0
Double Gate Road Pond	Spring-fed, artificial pond. Location: on farmland, near Davidsonville, Md.	Canada geese present October through April	Fresh water	2.1×10^7	6,200 0[f]	2.3×10^9	1.1×10^2	8.1×10^3 1.0×10^1	1.9×10^2 4.9×10^1
Fishing Creek Cove	Tidal cove; single inlet, 1 m wide. Location: Thomas Pt. (Chesapeake Bay) between South River and Fishing Creek	Whistling swans and, occasionally, ducks present from October through March	6–12	1.0×10^8	810 0[f]	2.7×10^{10}	2.7×10^2	9.1×10^3 3.1×10^3	3.1×10^1 2.3×10^1
Canninghouse Cove	Large shoal on north shore of Rhode River, located between shellfishing areas	Whistling swans, canvasbacks, and Canada geese present October through April	5–12	1.0×10^{10}	31,000 0[f]	6.2×10^{10}	6.2×10^0	1.7×10^2 1.3×10^1	1.0×10^0 2.2×10^2
Locust Point	Borders shellfishing area. Location: 2.5 miles from the mouth of the Rhode River, upstream from Canninghouse Cove	Waterfowl rarely present	5–12	1.7×10^9	1 0[f]	1.7×10^7	1×10^{-2}	5.0×10^1 1.1×10^2	1.0×10^0 8.0×10^0

[a] Volumes of water at the sampling sites were calculated from area, depth, and flow rate measurements (where applicable). Numbers of 100-ml volumes are presented to facilitate calculations of theoretical FC per 100 ml of water volume.

[b] Number of birds × numbers of hours in the field station; i.e., in the water.

[c] Theoretical fecal coliform input, bird hours × average load per capita per 24 h. (whistling swan, Canada geese, or swan/geese ratio output from Table 1 was derived from the waterfowl population at the respective sampling sites.)

[d] Theoretical FC input/number of 100-ml volumes calculated for the sampling site. These data provide an indication of the density of FC at each station expected to be recovered, based on waterfowl input alone (assuming an average 24-h die-off of the FC).

[e] Prior to introduction of birds into pond.

[f] Migration.

After dilution, the bird input amounted to 4.8 $\times 10^3$ FC per 100 ml of pond water per day.

The percentage of recoverable input compared with the calculated input was measured by sampling water and sediment at the stations on a routine basis. February 1976, when the waterfowl were roosting, and June 1976, when they had migrated, were sample sets selected for analysis of the effect of waterfowl on water quality. Values given in Table 2 reflect those of the other 16 sampling times, supporting the hypothesis that concentrations of waterfowl can cause an elevation in FC counts of surface water and sediment, viz., correlation coefficient $r = 0.79$, 95% confidence limits, for 18 of the sampling periods.

Before birds entered Lake Shore Pond, the most probable number of FC was 1/100 ml of surface water. After 220 bird hours, the count rose to 2,400/100 ml, or half the calculated daily input. The increase in the sediment FC population, approximately 2 logs higher, may have been caused by settling of particulate matter in the pond, which had an average depth of 1 m. Bacterial multiplication could also explain the increase in FC associated with the sediment samples. Despite the deterioration in water quality of the pond, measured by FC, neither *Salmonella* nor *Shigella* could be isolated from the water or sediment.

Double Gate Road Pond provided a freshwater study site. Although significantly more bird hours were spent at Double Gate Road Pond than at Lake Shore Pond, the FC populations at each station were similar since swans excreted almost 100 times more FC than geese (see Table 1). Swans and geese, however, both excreted uniformly large quantities of anaerobic bacteria, which very likely underwent multiplication or, at the least, survived in the bottom sediments of Double Gate Road Pond, which was relatively anoxic, i.e., ≤ 1 μg of dissolved oxygen per ml. Anaerobic conditions were enhanced by the large quantity of organic fecal matter from the extremely dense populations of geese. Approximately 5.1×10^5 anaerobic organisms were recovered, on the average, per g of sediment from Double Gate Road Pond, which was more than 1,000 times the concentration found at any of the other study sites (data not shown). Eighty percent of these were identified as *Clostridium* spp.

The greatest difficulty in equating bird output with recovery from the natural environment was the inability to control the variables, i.e., FC die-off (estimated to be, on the average, 24 h [12]), non-point source input, and water temperature. At sites included in the study that were not roosting areas for the birds, FC were much more numerous during May through October than during colder months when waterfowl could be expected to contribute to the FC populations. Thus, the impact of migratory waterfowl on the FC populations was minimized by the seasonality of waterfowl visits to the Bay.

At Canninghouse Cove, the largest site included in the study, located between the shellfishing areas of this part of the Bay, roosting waterfowl were calculated to add only 6.2 FC per 100 ml of water. This site is considered to be a model for river-bay ecosystems supporting dense migratory flocks. Ostensibly, 6.2 FC per 100 ml should not be highly significant when monitoring for water quality. However, during the bird season, sediment FC counts rose from 13 to 170 FC per 100 ml (130 FC per 100 ml in the nearby oyster beds), a sufficiently high population index to potentially result in restrictions being placed on shellfishing in these waters. FC sediment counts may, in fact, be a better indicator then surface water counts, since shellfish are bottom filter-feeders.

Locust Point provided a control site for the Canninghouse Cove area. Without populations of birds, this station yielded FC counts typical of other non-waterfowl roosting areas in the river; that is, the FC increased in numbers during the warm months of the year and decreased in the winter.

In summary, the data gathered during the course of this study provided evidence that healthy whistling swans and Canadian geese, migrating along the eastern U.S. Flyway, did not harbor detectable enteric, bacterial pathogens as a component of their natural flora. The study has also provided a method for quantitative prediction of FC loading, a non-point source loading, arising from migratory waterfowl populations. Such predications must, however, take into account the fact that FC densities can vary with different species of waterfowl and feeding habits.

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