Occurrence of *Escherichia coli* and Enterococci in *Cladophora* (Chlorophyta) in Nearshore Water and Beach Sand of Lake Michigan†

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Each summer, the nuisance green alga *Cladophora* (mostly *Cladophora glomerata*) amasses along Lake Michigan beaches, creating nearshore anoxia and unsightly, malodorous mats that can attract problem animals and detract from visitor enjoyment. Traditionally, elevated counts of *Escherichia coli* are presumed to indicate the presence of sewage, mostly derived from nearby point sources. The relationship between fecal indicator bacteria and *Cladophora* remains essentially unstudied. This investigation describes the local and regional density of *Escherichia coli* and enterococci in *Cladophora* mats along beaches in the four states (Wisconsin, Illinois, Indiana, and Michigan) bordering Lake Michigan. Samples of *Cladophora* strands collected from 10 beaches (*n* = 41) were assayed for concentrations of *E. coli* and enterococci during the summer of 2002. Both *E. coli* and enterococci were ubiquitous (up to 97% occurrence), with overall log mean densities (± standard errors) of 5.3 (± 4.8) and 4.8 (± 4.5) per g (dry weight). *E. coli* and enterococci were strongly correlated in southern Lake Michigan beaches (*P* < 0.001, *R*² = 0.73, *n* = 17) but not in northern beaches (*P* = 0.892, *n* = 16). Both *E. coli* and enterococci survived for over 6 months in sun-dried *Cladophora* mats stored at 4°C; the residual bacteria in the dried alga readily grew upon rehydration. These findings suggest that *Cladophora* amassing along the beaches of Lake Michigan may be an important environmental source of indicator bacteria and call into question the reliability of *E. coli* and enterococci as indicators of water quality for freshwater recreational beaches.

*Escherichia coli* is a widely used indicator of contamination originating from domestic sewage. High levels of this bacterium have been a chronic problem throughout southern Lake Michigan during summer months, resulting in numerous beach closures (19, 24, 34, 35). There is evidence that a significant portion of *E. coli* may arise from nonpoint sources originating within the beach area (e.g., birds, sand, and sediment storage) or from nearby inputs (riparian and wetland runoff) (16, 19, 26, 29, 32–34). Many beach closures challenge traditional paradigms since they are not associated with waste releases, recent rainfall, wind, or known pollution events. Bacterial multiplication both within the water column and along the shoreline has been suggested (2, 11, 29), but this idea has been questioned due to the limited availability of organic nutrients and adverse environmental conditions for survival of indicator bacteria, such as sunlight, temperature, and open beach desiccation (4, 10, 25; G. J. Medema, M. Bahar, and F. M. Schets, presented at the International Symposium on Health-Related Water Microbiology, Mallorca, Spain, 1997).

This study investigated the association and persistence of *E. coli* and enterococci in mats of the green alga *Cladophora* [almost exclusively *Cladophora glomerata* (L.) Kütz]. *Cladophora* is found in both fresh and marine waters worldwide (12). In the Great Lakes, *Cladophora* growing on rocks and other substrates in nearshore water can become detached and accumulate along the shoreline as large mats. The accumulation is common in bays with rocky substrates, particularly from June through September. These algal masses can result in offensive, malodorous conditions that may pose a public health risk. Although *Cladophora* is perennial, it tends to grow as an annual due to wintry conditions (5), and it is found primarily on shelving rocks and boulders (31). This alga provides shelter and nourishment to a wide variety of organisms, such as epiphytes (cyanobacteria and diatoms) and grazers (protozoa, mollusks, rotifers, and young crayfish) (8, 20, 30, 31).

The general assumption that traditional fecal indicators (e.g., *E. coli* and enterococci) do not occur in natural environments (soil or water) has recently been challenged. These bacteria occur in soils (6, 15, 16, 29) and riparian sediments (32) and perhaps as epiphytic microflora on terrestrial plants (22, 23, 28). Observations of these indicators living on aquatic plants, including algae, are lacking, but such associations would be significant, since aquatic macrophytes have the potential to harbor, shed, and possibly support the growth of these indicator bacteria. The presence of indicators associated with aquatic macrophytes may lead to the misinterpretation of water quality tests or misidentification of the source of indicator bacteria.

The specific objectives of this study were to (i) describe the relative association of *E. coli* and enterococci with floating, attached, and stranded *Cladophora*, (ii) characterize the regional distribution and density of these indicator bacteria in selected areas of Michigan, Indiana, Illinois, and Wisconsin, and (iii) determine whether *Cladophora* may act as a nonpoint source of *E. coli* and enterococci.

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In this paper, we refer to Cladophora algae that have become stranded on the beach, whether in mats or filaments, as “strands.” Detached algal strands submerged in the beach water are referred to as “floating” algae, whether they are within the water column or on the bottom. Finally, along the Sleeping Bear Dunes National Lakeshore, near Traverse City, Michigan was Washington Park Beach, Michigan City. Sites in Michigan included Municipal Beach, Waukegan; and 63rd Street Beach, Chicago. The site in Wisconsin included Bradford Beach, Milwaukee, and North Beach, Racine. The sites in Illinois were Illinois State Park Beach, Zion; Waukegan Municipal Beach, Waukegan; and 63rd Street Beach, Chicago. The site in Indiana was Washington Park Beach, Michigan City. Sites in Michigan included Good Harbor Bay, Sleeping Bear Bay, Platte Bay, and South Manitou Island along the Sleeping Bear Dunes National Lakeshore, near Traverse City.

**Site description.** All sites were sandy beaches with medium- to fine-grain sands, gradual slopes, and moderate wave exposure. Although there were domestic sewage sources near Bradford Beach, Waukegan Municipal Beach, and Washington Park Beach, most locations were not suspected to be directly or chronically impacted by sewage. Algal conditions varied substantially. At Bradford Beach, stranded algae occurred in large mats mixed with zebra mussels (*Dreissena polymorpha*) and were in an advanced stage of decomposition. At Illinois State Park Beach, thick mats of attached fresh algae were collected off riprap breakwaters within a cove. Most algal samples were 0.5 to 5 cm thick, except at Sleeping Bear Dunes National Lakeshore beaches, where algal mats were about 50 cm thick and several meters offshore. All of the beaches had the potential to accumulate large masses of *Cladophora*, given the appropriate season, wind, and currents.

**Sample collection.** *Cladophora* samples were collected between 24 June and 7 November 2002; more intensive sampling occurred during July 29 to 31. Samples from all locations were gathered from water, rock pilings, or beach sand. Algal samples were aseptically collected by hand and put into Whirl-Pak bags or glass jars. The samples were placed on ice and immediately taken to the laboratory. Air, water, and sand temperature were recorded at most of the locations. Samples from Indiana and Illinois were analyzed within 4 to 6 h of collection, but samples from the most distant locations were held for as long as 24 h at 4°C.

**Sand–lake water relationships.** Three randomly chosen transects were established on the beach at 63rd Street Beach and Washington Park Beach. Along each transect, two 0.5-m² quadrats were set in the water, 1 m from shore (nearshore); on the sand, 1 m inland from the shore (beach). All *Cladophora* within the quadrants were retrieved. A water sample was collected from each of the nearshore quadrants. Beach sand immediately underlying the strands in each beach quadrant was collected to a depth of 2 cm and placed in separate plastic bags.

**Microbiological analyses.** *E. coli* and/or enterococci were analyzed by membrane filtration (9). Generally, undiluted lake water samples were filtered in volumes ranging from 10 to 100 ml. For analyzing algae and sand samples, an initial elution step was necessary to release the bound bacterial cells. One gram portions of homogenized algal samples were weighed and placed in sterile 15-ml centrifuge tubes, to which 9 ml of sterile phosphate-buffered diluent water (PBW) (pH 6.8) was added. The algal-PBW mixture was vigorously shaken for 2 min and centrifuged briefly (45 s) at 2,000 rpm (653 × g) to allow the large particles to settle. If necessary, the supernatant was further diluted in PBW, and appropriate volumes ranging from 1 to 30 ml were filtered. Filters were placed on thermotolerant-*E. coli* medium (mTEC) or enterococcal medium (mE) and incubated at 44.5°C (*E. coli*) or 41°C (enterococci) (9). Sequential rinsing of algae showed that an average of 55% of bacteria were recovered with this technique (data not shown).

Fifty grams of representative sand was added to 100 ml of PBW. The mixture was shaken for 2 min and allowed to settle for 30 s. The supernatant was serially diluted, and appropriate volumes were analyzed for *E. coli* and enterococci as previously described. Fresh (wet) samples of sand or algae were dried at 108°C for 24 h to determine dry weight. All bacterial concentrations in algae or sand are expressed in grams (dry weight) unless otherwise noted. Sequential rinsing showed that a mean range of 86 to 100% of bacteria was recovered from the sand-algae mixture using this technique.

**Sampling locations: regional surveys.** A survey was conducted along 10 beaches on Lake Michigan located in Wisconsin, Illinois, Indiana, and Michigan to determine the relative abundance of *E. coli* and enterococci within *Cladophora* (Fig. 1). The sites in Wisconsin included Bradford Beach, Milwaukee, and North Beach, Racine. The sites in Illinois were Illinois State Park Beach, Zion; Waukegan Municipal Beach, Waukegan; and 63rd Street Beach, Chicago. The site in Indiana was Washington Park Beach, Michigan City. Sites in Michigan included Good Harbor Bay, Sleeping Bear Bay, Platte Bay, and South Manitou Island along the Sleeping Bear Dunes National Lakeshore, near Traverse City.

**RESULTS**

**Regional surveys.** *E. coli* densities in *Cladophora* for the 10 beaches surveyed were generally high but highly variable; the overall log mean *E. coli* density was 5.3 ± 4.8 CFU/g. The geometric log mean density of *E. coli* in these algae was lower, 3.99 CFU/g, and the median was 3.72 CFU/g. The highest *E. coli* counts were found in attached *Cladophora*: log 6.2/g in

![Image](52x524 to 294x722)
epilithic algae at North Beach. Attached algae at Waukegan Municipal Beach and Washington Park Beach also had higher concentrations than local unattached algae. A Kruskal-Wallis test showed no significant difference in means among attached, floating, and stranded algae ($P = 0.067$). High variance and small sample size made examination of these differences difficult. Algae collected from beaches of South Manitou Island, Platte Bay, 63rd Street Beach, and Bradford Beach had relatively low E. coli counts (Fig. 2A).

Concentrations of enterococci in Cladophora averaged $4.8 \pm 4.5 \log$ CFU/g. As with E. coli concentrations, geometric mean and median concentrations of enterococci were much lower ($2.3$ and $2.1 \log$ CFU/g), suggesting nonnormal distribution of bacteria among samples. The highest enterococci concentrations were found in floating algae at North Beach ($6.0 \log$ CFU/g). Very low counts of enterococci were recovered from algae collected at South Manitou Island and Good Harbor Bay; also, fewer enterococci were recovered at Bradford Beach (Fig. 2B). In general, concentrations of enterococci were higher along southern Lake Michigan, particularly at North Beach, Waukegan Municipal Beach, Illinois State Park Beach, and Washington Park Beach. Median enterococcus counts for stranded, floating, and attached algae were $1.9$, $2.1$, and $4.97 \log$ CFU/g. The Kruskal-Wallis statistical test implied that concentrations of enterococci in attached algae were significantly higher than those in floating or stranded algae ($P = 0.025$). Variation in indicator bacteria among beaches is difficult to explain due to limited sample size.

Algal E. coli was correlated with enterococci in southern Lake Michigan beaches ($P < 0.001$, $R^2 = 0.73$, $n = 17$) but not northern beaches ($P = 0.892$, $n = 16$). In general, Cladophora of southern Lake Michigan tended to have higher concentrations of E. coli and enterococci. Lake water followed similar trends ($19$, $32$, $34$).

**Sand versus lake water.** Transect sampling suggested that patterns of E. coli concentrations in water, beach sand, and Cladophora (floating and stranded) were similar at Washington Park Beach and 63rd Street Beach. In the combined data, E. coli counts in floating algae were significantly higher than in stranded algae, sand, or water, and stranded algae had more E. coli than either sand or water ($P \leq 0.05$). Algal E. coli counts from Washington Park Beach were higher ($5.3 \pm 4.7 \log$ CFU/g) than counts from 63rd Street Beach ($4.7 \pm 3.9 \log$ CFU/g) ($P < 0.006$), even though 63rd Street Beach historically had higher water E. coli counts (Chicago Park District and LaPorte Health Department, Indiana, unpublished data). E. coli in stranded algae and adjacent water were correlated ($P = 0.024$, $n = 6$), but the small sample size makes this inference tenuous.

E. coli concentrations in floating algae and lake water at Washington Park Beach were significantly correlated ($P = 0.004$, $R^2 = 0.72$, $n = 9$). Similarly, there was a significant relationship between water temperature (that ranged between 6 and 23°C during June to November) and E. coli concentrations in floating algae ($P = 0.004$, $R^2 = 0.41$, $n = 18$). The highest E. coli density occurred during the middle of summer. It is noteworthy that algal E. coli concentrations remained relatively stable during most of the summer; the log mean E. coli concentration from June 24 through September 13 was $4.0 \pm 0.33$ CFU/g. By October, algal E. coli density was below detection, coinciding with a drop in water temperature, even though Cladophora still looked healthy.

**E. coli persistence in Cladophora mats.** While there was generally an exponential decline in E. coli over a 27-h sunlight exposure period, only a modest population loss occurred in the first 9 h, even in the 1-mm-thick mat (Fig. 3A). Without replication, it is difficult to generalize, but there is a preliminary suggestion of an increase in density in the 6-mm mat over the
first 9 h, coincident with a mean algal high temperature of 48°C. *E. coli* counts in 1- and 2-mm mats quickly declined; in 1-to 4-mm-thick mats, the counts remained at about 2 log CFU even after 27 h of exposure. Mats of 6-mm thickness were much more resistant to depopulation, maintaining their density at almost 4 log after 27 h.

**Enterococcal persistence in Cladophora mats.** Enterococci appeared to be as vulnerable as *E. coli* to exposure at algal thickness of 1 mm (Fig. 3B). Enterococci in 2-, 4-, and 6-mm mats remained at about 4 log CFU/g during the first 9 h. For mat thicknesses of 2 and 4 mm, enterococcal counts declined by approximately 2 log in 18 h of exposure but then remained steady thereafter. Enterococci densities in the 6-mm mat were essentially unaffected throughout the exposure time.

**Indicator bacterial survival and growth potential in sunlight-exposed and refrigerated Cladophora mats.** Concentrations of both *E. coli* and enterococci increased by approximately 4 log in 24 h following rewetting of the dried *Cladophora* mat (Fig. 4). During the next 72 h, counts of these bacteria remained stable (enterococci) or declined only slightly (*E. coli*). When the experiment was terminated after 96 h, concentrations of *E. coli* and enterococci were still in excess, by 2 log, of their initial numbers. These results suggest that *E. coli* and enterococci could persist for long periods in the sun-dried and subsequently refrigerated *Cladophora* mats; the residual bacteria in the dried algae could readily multiply upon hydration and incubation at 35°C.

**DISCUSSION**

Associations between *Cladophora* and microbial communities are not well understood, although some research has presented evidence of a relationship between *Cladophora* and bacilliform bacteria (27). The cell wall of *Cladophora* provides a suitable attachment and grazing surface for many other organisms, such as diatoms, protozoa, mollusks, rotifers, and young crayfish (8, 27, 31), and links between bacteria and algae have been found frequently in aquatic environments (3, 13, 18, 21). The findings of this study are significant because it is perhaps the first to demonstrate the presence of fecal indicator bacteria, *E. coli* and enterococci, on *Cladophora*.

In spite of a limited number of studies that suggest that indicator bacteria (*E. coli* and enterococci) can multiply in

![FIG. 3. Responses of *E. coli* (A) and enterococci (B) within *Cladophora* mats that were exposed to direct sunlight for four consecutive days. Algal mats of various thicknesses were retrieved and analyzed after 9, 18, and 27 h of exposure.](image1)

![FIG. 4. Response of *E. coli* and enterococci (± 1 standard error) in rehydrated, unaugmented, and unseeded *Cladophora*, which had been sun dried (for 28 h) and subsequently stored at 4°C for over 6 months.](image2)
limiting factors include interspecific competition, predation, and nutrient limitation (6, 10). The present study demonstrates that Cladophora harbors high densities of E. coli and enterococci relative to water and beach sand and that the indicator bacteria in Cladophora are ubiquitous and perhaps even independent of point sources. Further, the experiments show that E. coli and enterococci can survive for extended periods (over 6 months) in the algal mat and quickly multiply when moisture is returned. Thus, Cladophora stranded on the beach is a potential source of these indicator bacteria whether the algal mat is dry or remains moist or whether it has been exposed to sunlight or buried in the sand. These observations demonstrate that Cladophora provides both the minimal habitat and nutrient source for survival and possibly growth of E. coli and enterococci.

The explanation for the occurrence of bacteria in floating and attached algae is less intuitive. Perhaps Cladophora is so rich in nutrients and biofilm habitat that indicator bacteria can maintain populations despite obvious interspecific pressures from resident organisms, such as periphyton and grazers (8, 20, 27, 31). Regardless, the persistence and survival of indicator bacteria in Cladophora under natural conditions seems to depend on a variety of factors (predation, sunlight, and temperature) (10). Since E. coli and enterococci survived for over 6 months in sun-dried and refrigerated Cladophora, perhaps other factors (competition, predation, and sunlight) were responsible for the gradual disappearance of E. coli and enterococci in naturally occurring Cladophora by October.

Our findings clearly suggest that Cladophora can be a secondary habitat for indicator bacteria that could potentially influence water quality in affected Great Lakes swimming areas. The long-term survival of E. coli and enterococci in Cladophora mats also has important ecological and public health implications. Masses of floating Cladophora, as a result of wave action, can release indicator bacteria and elevate their levels in the water. Also, algal mats washed onto beach sand may get buried in the sand by wave action or human activities, where they are protected from sunlight and desiccation. Here, indicator bacteria may multiply due to available nutrients from the decomposing mats; in turn, the beach sand can serve as a source of indicator bacteria for the nearshore water, especially when waves resuspend buried mats. Previously, studies have shown that pathogenic bacteria (e.g., vibrios) are often associated with algae (17). It is possible that Cladophora provides a niche for pathogenic bacteria.

While the case for natural multiplication needs further validation, Cladophora can be a reservoir for E. coli and enterococci in Lake Michigan. To understand the ecological and environmental implications of the present findings, more laboratory studies are necessary. These might include (i) in vitro studies showing the range of tolerance and growth potential of subject bacteria under a variety of environmental conditions (insolation, desiccation, and temperature), (ii) a thorough investigation of the genomic and phenotypic relationships of algae and ambient bacteria to investigate clonality or source-sink relationships further, (iii) noninvasive sterilization and inoculation of algae using wild and lab strains to discover intrinsic growth potential, maximum carrying capacity, and associated limiting factors, (iv) high-resolution microscopic studies of algal thalli and biofilm to further understand the physical association of algae and indicator bacteria, and (v) more investigations of the health implications of these findings.

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REFERENCES


nities associated with Bangia atropurpurea and Cladophora glomerata in west-

erlands.
20. Marks, J. C., and M. E. Power. 2001. Nutrient induced changes in the species composition of epiphytes on Cladophora glomerata Kutz (Chlorophyta). Hy-
lation and phylogenetic characterization of bacteria capable of inducing