Seasonal Variability and Transport of Suspended Microfungi in a Southeastern Salt Marsh†

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Tidally induced fluctuations and transports of microfungi were investigated. Samples were collected at three depths from three stations positioned at a transect in a large salt marsh creek. Samples were taken every 1.5 h for 50 consecutive h during neap tides and 50 consecutive h during the corresponding spring tides. In each season, microfungi concentrations fluctuated out of phase with the tides during both neap and spring tides. Mean concentrations of suspended microfungi did not vary appreciably throughout the year. Fungi were exported from the marsh during the majority of the tidal cycles studied. The results suggest that microfungi may serve as indicators of water mass movements.

Several papers have been published dealing with the use of microorganisms to differentiate between coastal and open waters or as indicators of mass water motion (4, 5, 20). Microorganisms may be particularly useful as indicators in salt marshes, where only slight differences in salinity of water masses preclude the use of salinity-tracing techniques, and they may also differentiate between material entering an estuary (or salt marsh) and material resuspended by physical forces of water motion (4, 5). Microorganisms as indicators of water masses in coastal environments must meet certain criteria: they must move passively with a water mass, remain viable under salinity stress, and be enriched (either total numbers or a given species) in one water mass relative to another.

Bacteria appear to have limited applicability as such indicators. Vaatanen (20) reported that the difference between bacterial populations of inshore and offshore waters is in population composition rather than numbers. Wilson et al. (21), studying the waters of a tidal inlet, reported findings similar to those of Vaatanen. They found no change in total bacterial numbers over the course of a tidal cycle, but cells suspended in flooding waters were physiologically different from cells in ebbing waters. Wimpenny et al. (22) suggested tracing water motion in rivers by introducing foreign, easily identifiable bacteria or phages. Fecal bacteria such as Escherichia coli have some use as indicators, but their use as indicators is confined to polluted waters and is limited by low viability in saline waters.

Microfungi are potentially useful as indicators of water mass movements and resuspension in estuaries and salt marshes. Resuspended saprophytic microfungi move passively with a water mass, survive salinity stress (16), and are enriched in a water mass entering an estuary by river input (4, 18) and leaving a salt marsh by tidal flushing (1, 2).

Despite this potential, few studies are available demonstrating the usefulness of microfungi as indicators of water masses and resuspension. Cooke's (4) study of microfungi dispersal in the Thames River Estuary, N.Y., demonstrated diluting and mixing of salt and freshwater. Tidally induced oscillations in microfungi concentrations in a southeastern salt marsh were attributed to resuspension from the intertidal zone by Chrzanowski and Stevenson (1, 2). Recently, Stevenson et al. (19) used suspended microfungi to investigate the extent of seawater intrusion into a salt marsh.

Continued development of the concept of microfungi as indicators of water movements and resuspension in salt marshes requires an assessment of both tidally induced and seasonal variations of suspended microfungi concentrations (4). Additionally, before microfungi can be used to trace a water mass after it exits a marsh, we must demonstrate that the exiting water is also transporting microfungi. The work reported herein addresses these two concerns.

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**MATERIALS AND METHODS**

Sampling was done at a transect across Town Creek in the North Inlet, S.C., ecosystem (latitude, 33°20' N; longitude, 79°10' W). Town Creek is a major marsh creek and forms a large section of the inlet. Descriptions of North Inlet and the sampling location were previously published (2, 6, 14).

Samples were collected seasonally throughout 1979 from three boats moored and positioned so that data would accurately reflect material flow through the transect (3, 13). Water was pumped with a Guzzler pump (Dart Union Corp.) from 0.2 m below the surface, 0.2 m above the bottom, and a midway point; collected in sterile, acid-washed, 500-ml glass bottles; placed on ice in the dark; and transported immediately to laboratory facilities onshore. The pump intake tubing was autoclaved between each 50-h sampling period. Samples were collected once every 1.5 lunar h (95 min) for approximately 50 consecutive h (four tidal cycles) during neap tides and spring tides. During the winter, samples were taken during a mid tide instead of a neap tide.

Microfungi (any spore, microscopic mycelial mass, or hyphal fragment) were enumerated by the method ofChrzanowski and Stevenson (2). Triplicate subsamples of at least 2 ml were filtered onto membrane filters (type HA; Millipore Corp.) held in sterile filtration funnels (Gelman Sciences, Inc.). Filters were removed to Martin medium (15) and incubated in the dark at room temperature (25°C) for 5 days. External contamination was avoided by covering air conditioner openings and doorways with antiseptic-dampened gauze and using an antiseptic foot bath and an atmospheric microparticle filter (Prime Aire, model 1120; Dexon Inc.). Mean values from triplicate plaatings were used in subsequent computations.

Water velocity was measured concurrently with sample collection. These data were computer fitted to a smooth curve, and new values were extrapolated at every one-tenth depth from surface to bottom (3, 11).

The instantaneous transport of microfungi at each sampling interval was calculated from the following equation:

\[
F = \rho \sum_{j=1}^{N} \left(0.5 V_{oj} W_{oj} C_{oj} + \sum_{i=1}^{N} V_{ij} W_{ij} C_{ij}\right),
\]

where \(F\) is the transport of microfungi, \(\rho\) is the water density (1.02 g/ml), \(N\) is the number of sampling stations, \(h_j\) is the depth at station \(j\), \(V_{oj}\) is the current velocity, \(W_{oj}\) is the width of the sampling station, and \(C_{oj}\) is the concentration of microfungi at the surface of station \(j\). Similarly, \(V_{ij}, W_{ij},\) and \(C_{ij}\) are the velocity, width, and concentration, respectively, of microfungi at depth \(i\) of station \(j\) (13).

The net directional transport of microfungi was calculated by fitting the instantaneous transport values (\(F\); equation 1) to a descriptive equation. The equation fits instantaneous transport as a function of time (T, hours from an arbitrary starting point) and calculates a mean net transport (\(\mu\), propagules per second) for each four consecutive tidal cycles. The descriptive equation is:

\[
F = \mu + \alpha_1 \sin(2\pi T/24.84) + \beta_1 \cos(2\pi T/24.84) + \alpha_2 \sin(2\pi T/12.42) + \beta_2 \cos(2\pi T/12.42) + \epsilon,
\]

where \(\alpha_1, \alpha_2, \beta_1, \beta_2,\) and \(\epsilon\) are coefficients and \(\epsilon\) is a random error term. Instantaneous transports are a result of the tide, so tidal periods (24.84, 12.42, and 6.21 h) were included in the equation to explain deviations from the mean net transport (\(\mu\)). Sine and cosine terms explain variability resulting from tidal oscillations and reduce the standard error of the estimator \(\mu\), allowing for a statistical test of significance. The equation is a form of the general linear model, and least-squares estimates of \(\mu\) and coefficients were found by using the SAS (Statistical Analysis Systems) computing package (10).

**RESULTS**

A single pattern was observed in the fluctuations of suspended microfungi during the 32 tidal cycles monitored throughout the year. Concentrations of microfungi fluctuated out of phase with the tide, maximum concentrations occurred at low tide, and minimum concentrations occurred at high tide. Examples of typical fluctuations are shown in Fig. 1A and 2A. Occasionally, the maximum concentration of microfungi suspended at low tide corresponded to maximum tidal amplitude; as tidal amplitude decreased on successive tidal cycles, so did the concentrations of microfungi. The standard error of the mean microfungi concentrations was less than 10% of the mean for more than 90% of the sampling periods (error bars omitted in figures) and reflects low station-to-station variability.

Descriptive statistics and seasonal mean microfungi concentrations are shown in Table 1. The highest mean concentrations were during the winter, mid, and spring tides. Mean concentrations for both spring (May) sampling periods and the summer neap tide sampling were similar; however, there was a wider range in the summer data. The mean concentration during the summer spring tide was nearly twice the mean for the corresponding neap tide, although the ranges were similar. The largest difference between concentrations of suspended microfungi for neap and spring tides was during the fall, with \(7.3 \times 10^6\) propagules per m³ during the spring tide, nearly four times the neap tide concentration. Apart from the fall, ranges in concentrations were similar between neap and spring tides for each season. The highest seasonal mean concentration was in the winter and nearly double the mean for any other season (Table 1).

Typical transport patterns of microfungi are shown in Fig. 1B and 2B. The magnitudes of export far exceeded those of import. During the winter mid tide, few data points fell appreciably below zero (import). Net exports during the spring (May) were similar to those of the winter and ranged from \(1.4 \times 10^8\) to \(1.6 \times 10^9\) propagules per s. The second tidal cycle of the spring tide was the only period in spring when fungi were imported. No instances of net import were
recorded during the summer, and maximum transport rates were similar for each tidal cycle. Two instances of net import occurred in the fall, during the final tidal cycle of each series.

Average transports for each tidal series (four tidal cycles) were calculated by using equation 2. The descriptive equation accurately reproduced the observed transport patterns, and a comparison of described and measured transports generated correlation coefficients ($r^2$) that ranged from 0.77 to 0.94 (Table 2). Microfungi were exported during each tidal series, with the highest transport rate, $27 \times 10^8$ propagules per s, during the winter spring tide. The lowest export rate, $2 \times 10^8$ propagules per s, was calculated for the fall neap tide and corresponded to the period of lowest fungi concentrations. Except for the fall spring tide, mean net transports for each tidal series were significantly different from zero transport at $P$ less than 0.05 (two-tailed $T$ test). The highest seasonal transport rate (averaged tidal series transports), $9.6 \times 10^{13}$ propagules per cycle, was during the winter, with summer, spring, and fall following in order.
TABLE 1. Descriptive statistics for data collected during each sampling period

<table>
<thead>
<tr>
<th>Season (date)</th>
<th>Tidal series</th>
<th>N</th>
<th>No. (×10^6) of Fungal propagules per m^3</th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Minimum value</td>
<td>Maximum value</td>
<td>Range</td>
<td>Seasonal mean</td>
<td></td>
</tr>
<tr>
<td>Winter (Feb.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–23</td>
<td>Mid</td>
<td>1,056</td>
<td>9.3</td>
<td>0.3</td>
<td>28.9</td>
<td>28.6</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>25–27</td>
<td>Spring</td>
<td>1,056</td>
<td>8.2</td>
<td>0.8</td>
<td>29.8</td>
<td>29.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring (May)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–20</td>
<td>Neap</td>
<td>1,056</td>
<td>3.6</td>
<td>0.2</td>
<td>14.5</td>
<td>14.3</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>25–27</td>
<td>Spring</td>
<td>1,056</td>
<td>3.8</td>
<td>0.1</td>
<td>15.3</td>
<td>15.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer (July)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17–19</td>
<td>Neap</td>
<td>1,056</td>
<td>3.1</td>
<td>0.0^a</td>
<td>23.5</td>
<td>23.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>10–12</td>
<td>Spring</td>
<td>1,056</td>
<td>5.9</td>
<td>0.0</td>
<td>27.8</td>
<td>27.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall (Oct.–Nov.)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26–28</td>
<td>Neap</td>
<td>1,056</td>
<td>1.9</td>
<td>0.0</td>
<td>8.6</td>
<td>8.6</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>Spring</td>
<td>1,056</td>
<td>7.3</td>
<td>0.2</td>
<td>37.9</td>
<td>37.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Below detectable limits.

DISCUSSION

Kjerfve et al. (12) reported that the North Inlet marsh system is covered by tidal waters approximately 30% of the year; however, the extent of submergence varied over the course of the year, with only 27% covered in the winter (January) and 42% covered in the fall (October). Based on the extent of floodings, we would expect the lowest concentrations of resuspended microfungi to occur during the winter and the highest to occur during the fall. Generally, mean concentrations of microfungi did not vary appreciably over the year; however, the highest concentrations were during the winter. High winter concentrations may be anomalous, since just before sampling, there was a winter storm with a heavy snowfall and a rapid melt. During this sampling period, the discharge of water from Town Creek was nearly 50% greater than during any other season. High microfungi concentrations probably reflect runoff from the forested areas bordering the marsh. Apart from the winter, the highest concentrations occurred during the fall spring tides and only partially reflect the extent of marsh submergence. Cooke (4), working in a riverine system, reported high fungi concentrations during the late fall through spring and attributed the levels to precipitation and increased river discharge. Despite variability due to weather conditions, the microfungi concentrations were

TABLE 2. Mean net export of microfungi for each tidal series, tidal cycle, and season

<table>
<thead>
<tr>
<th>Season (date)</th>
<th>Tidal series</th>
<th>r^2</th>
<th>P &gt; T/</th>
<th>Export of fungi</th>
<th>Mean net (10^6 Propagules per s ± SE)</th>
<th>(10^13 Propagules per cycle)</th>
<th>Seasonal avg (10^13 Propagules per cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (Feb.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>21–23</td>
<td>Mid</td>
<td>0.82</td>
<td>0.004</td>
<td></td>
<td>16.2 ± 5.1</td>
<td>7.2</td>
<td>9.6</td>
</tr>
<tr>
<td>25–27</td>
<td>Spring</td>
<td>0.79</td>
<td>0.003</td>
<td></td>
<td>26.8 ± 8.0</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Spring (May)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–20</td>
<td>Neap</td>
<td>0.84</td>
<td>0.005</td>
<td></td>
<td>8.1 ± 2.6</td>
<td>3.6</td>
<td>2.9</td>
</tr>
<tr>
<td>25–27</td>
<td>Spring</td>
<td>0.83</td>
<td>0.023</td>
<td></td>
<td>4.9 ± 2.1</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Summer (July)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17–19</td>
<td>Neap</td>
<td>0.94</td>
<td>0.0001</td>
<td></td>
<td>8.3 ± 1.2</td>
<td>3.7</td>
<td>6.4</td>
</tr>
<tr>
<td>10–12</td>
<td>Spring</td>
<td>0.79</td>
<td>0.004</td>
<td></td>
<td>20.1 ± 6.2</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Fall (Oct.–Nov.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26–28</td>
<td>Neap</td>
<td>0.83</td>
<td>0.032</td>
<td></td>
<td>2.3 ± 1.0</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>2–4</td>
<td>Spring</td>
<td>0.77</td>
<td>0.293</td>
<td></td>
<td>6.3 ± 5.8</td>
<td>2.8</td>
<td></td>
</tr>
</tbody>
</table>
were relatively consistent from season to season and similar to levels reported by Stevenson et al. (19).

Tidally induced variability was more dramatic than seasonal variability. In each season, during neap or spring tides, microfungi concentrations were out of phase with the tidal rhythm, and mean concentrations could range ninefold over a 6-h period. The rhythmic fluctuation pattern, keyed to tidal action, appears to be characteristic of sampling locations near marsh inlets (2). Stevenson et al. (19) reported tidally induced fluctuation patterns at a marsh-ocean interface, whereas simultaneous sampling at a high marsh location failed to detect a similar pattern. They suggested that high concentrations of suspended microfungi at low-tide periods were the result of movement of a microfungi-enriched parcel of water from high marsh to low marsh areas. The transport data of this study lends credibility to the speculations of Stevenson et al. (19), as microfungi were transported out of the marsh during each season, and average rates were statistically different from a net zero transport.

The data presented here indicate that seasonal variations in the concentrations of suspended microfungi should not limit their usefulness as water mass indicators in salt marsh environments. Additionally, the recurrent, tidally induced, out-of-phase fluctuations indicate resuspension of detrital material and suggest that the controlling mechanism is similar throughout the year. Greater than 90% of the tidal cycles monitored indicated a net movement of microfungi out of the marsh. Microfungi should, therefore, be useful to trace a water mass after it has exited a marsh.

The source of the enumerated microfungi, an important aspect of this and similar studies, has yet to be addressed. In light of reports of extensive colonization of standing and dead Spartina by marine and terrestrial saprophytic fungi (7–9) and a recent report that fungal biomass can equal 20 to 50% of the dry weight of Spartina leaves (17), it seems reasonable that the cultivated propagules were the result of hyphal fragments contained within small bits of detritus. If this is the case, microfungi may be a useful tool in studies of sediment-water column coupling. Further studies are necessary to determine the source of the suspended microfungi and their possible role in decomposition of detrital material.

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LITERATURE CITED


