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Indicators of airborne fungal concentrations in urban homes: Understanding the conditions that affect indoor fungal exposures

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HIGHLIGHTS

- We measured indoor airborne fungal types and levels in urban Syracuse, NY homes.
- We developed models predicting fungal exposure from home characteristics.
- High fungal levels were related to visible mold, musty odors and cockroaches.
- High fungal levels were also related to outdoor concentrations.
- Snow cover increased indoor/outdoor fungal ratios.

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ABSTRACT

Indoor fungal exposure can compromise respiratory health. Low-income urban areas are of concern because of high asthma and allergy rates and housing disrepair. Understanding the conditions that affect indoor fungal exposures is important for assessing health risks and for developing mitigation strategies. We examined the types and concentrations of airborne fungi inside and outside of homes in low-income areas of Syracuse, NY as well as the effect of snow cover on fungal levels. At 103 homes, air samples for viable fungi were collected, occupants were interviewed and homes were inspected for visible mold, musty odors, water problems and other factors. Multivariable logistic regression was used to relate high fungal levels to home conditions. Predominant indoor fungi included *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria* and hyaline unknowns. Basidiomycetes and an uncommon genus *Acrodontium* were also found frequently due to analysis methods developed for this project. With snow cover, outdoor total fungal levels were depressed and indoor concentrations were three times higher than outdoor on average with a maximum of 29 times higher. Visible mold was related to elevated levels of *Penicillium* (OR 4.11 95% CI 1.37–14.0) and bacteria (OR 3.79 95% CI 1.41–11.2). Musty, moldy odors were associated with elevated concentrations of total fungi (OR 3.48 95% CI 1.13–11.6) and basidiomycetes. Cockroaches, an indicator of moisture, were associated with elevated levels of *Penicillium* (OR 3.66 95% CI 1.16–13.1) and *Aspergillus* (OR 4.36 95% CI 1.60–13.4). Increasing relative humidity was associated with higher concentrations of *Penicillium*, yeasts and basidiomycetes. Visible mold, musty odors, indoor humidity and cockroaches are modifiable factors that were important determinants of indoor fungal exposures. Indoor air investigators should interpret indoor:outdoor fungal ratios cautiously when snow cover is present.

1. Introduction

The increased prevalence of childhood asthma and allergies has focused attention on the exposure of young children to dampness and mold in homes. Low-income, urban areas are of particular concern because childhood asthma and allergy rates are higher there and respiratory problems may be more severe (Aligne et al., 2000; Gold and Wright, 2005). In addition, low-income housing is often of poor quality

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and deteriorated, presenting conditions that are conducive to pest infestations and fungal growth (Breysse et al., 2005; Rauh et al., 2002). Mold and dampness are common problems in the United States affecting an estimated 10–50% of all homes (Mudarri and Fisk, 2007; U.S. Census Bureau, 2011). Reviews of the worldwide literature have concluded that exposure to damp and moldy indoor environments is associated with health problems such as wheeze, cough, upper respiratory symptoms and asthma (Bornehag et al., 2001; Institute of Medicine, 2004; Mendell et al., 2011). Although dampness and mold are common and known to be related to adverse respiratory health effects, far less is known about the specific agents responsible for causing these problems (World Health Organization Europe, 2009). Quantitative measurements of fungi and other microbiological factors would seem to be of obvious importance for identifying causative agents; however, results have been inconsistent. Measured concentrations of specific fungal taxa (Gent et al., 2012; Pongracic et al., 2010; Reponen et al., 2012; Salo et al., 2006; Stark et al., 2005), fungal indicators and other microbiological factors (Celedon et al., 2007; Dales et al., 2006; Gillespie et al., 2006) have been linked to an increased risk of respiratory symptoms or other health effects. At the same time, inverse associations have been reported, suggesting a protective role for some of these factors (Behbod et al., 2015; Douwes et al., 2006; Karvonen et al., 2012; Sordillo et al., 2010). Given this uncertainty, further work is warranted.

Many epidemiology studies have measured exposure to dampness and mold using indirect methods such as questioning occupants or by an inspector's observation of home conditions e.g. (Antova et al., 2008; Fisk et al., 2007; Gunnbjornsdottir et al., 2006; Simoni et al., 2005; Zock et al., 2002). Because fungal growth requires moisture, conditions such as leaking water, damp stains, excessive condensation, and high indoor humidity have potential to support fungal growth and contribute to airborne fungal exposures. Similarly, observations like visible mold and musty smells suggest a clear potential for airborne fungal exposures. The presence of carpeting may act as a potential source for indoor microbial exposure, where soil and plant debris tracked in from outdoors could accumulate and allow microbial growth and spore release from carpets (Bischof et al., 2002; Dharmage et al., 1999). Alternatively, carpets may act as potential sinks that trap and curb fungal growth and dispersal. Cats or dogs in the home can potentially transport fungi from outdoor soil and plant debris (Chew et al., 2003). Cat litter boxes have also been hypothesized as sources of moisture capable of supporting fungal growth (O'Connor et al., 2004). The observation of cockroaches may suggest that water and moisture sources are present and sufficient to support infestation (Portnoy et al., 2013). Home crowding and lack of ventilation can contribute to condensation problems. Poor upkeep, rodent infestation, and lack of sanitation each may suggest some potential for fungal growth and exposure in the home.

However, conditions that potentially contribute to exposure are not the same as actual exposure. The observation of water damaged materials or musty odor is not necessarily indicative of the presence of unusually high levels of fungal spores. Water damaged materials that have dried may not be supporting the current microbial growth. Musty odors are due to volatile organic compounds which can originate from sources other than fungi such as carpeting and furnishings (Kim et al., 2007) as well as from animals, both pets and pests. Measuring the types and concentrations of airborne fungi provides a means of assessing exposure more directly than through visual observation alone. As the relationships between fungal exposures and health effects continue to emerge, it is important to understand the home characteristics and conditions that give rise to these exposures, Understanding the conditions that predict indoor airborne fungi is important for developing effective interventions for controlling fungal exposure. Because the distribution of fungal types and levels varies by geographic location and climate, multi-year and seasonal information for a specific area provides valuable baseline and comparative data for public health and indoor air quality investigators in that region.

Direct measurements of fungi have several limitations that must be taken into account. Because fungi are naturally present in outdoor and indoor air in virtually all environments, there are no guidelines for typical or safe exposure levels. Outdoor airborne fungal spores readily infiltrate indoor spaces through windows, doors, ventilation systems and other openings in the building envelope so that interpretation of indoor air sampling results requires some consideration of the corresponding outdoor fungi (Dillon et al., 1996). Seasonal differences may also be important, with greater microbiological activity and higher fungal levels expected during warm weather. In cold climates, fungal concentrations can be affected by the presence of snow cover which may depress spore release by blanketing soil and plant matter (Li and Kendrick, 1995; Macher, 1999; Reponen et al., 1992). The choice of air sampling method influences exposure estimates because there is no single method that can comprehensively characterize airborne fungi. Non-viable, spore trapping methods facilitate recovery of spores, however many spores cannot be identified by direct microscopy. Culture-based methods facilitate the identification of spores but underestimate total spore levels because only spores that are viable and that grow on the chosen culture medium are counted (Dillon et al., 1999). The expertise of laboratory personnel also affects identification of spores. Air samples may not be representative of exposure because of the short sampling periods required to avoid over-loading sampling media. Finally, a lack of standardized methods for sample collection, analysis and reporting hinders comparisons between studies. Nonetheless, when these concerns are addressed in an epidemiology study, air sampling for fungi can contribute to greater understanding of the specific agents and exposures in indoor environments and their relationship to residential conditions.

In a previous study, we determined that exposure to high levels of indoor *Penicillium* was significantly associated with wheezing episodes among infants living in low-income urban homes in Syracuse, NY (Rosenbaum et al., 2010). In this study, we report further on the types and concentrations of fungi in the indoor and outdoor air. We examined the relationship between measured levels of indoor airborne fungi and home conditions and developed models predicting fungal exposure from these home conditions while considering important sources of variability. Frequent snowfall in this region allowed us to assess the effect of snow cover on indoor and outdoor fungal concentrations. We report on indoor to outdoor fungal ratios with snowfall because indoor air practitioners frequently use such ratios to assess building mold problems. This subject is not well documented in the literature and has not focused on temperate climates with episodic snowfall.

2. Methods

2.1. Project and cohort description

This project was part of a larger birth cohort study designed to examine the relationship between indoor environmental pollutants and infant wheezing in a low-income, urban setting. The AUDIT study (Assessment of Urban Dwellings for Indoor Toxics) was approved by the institutional review board of the State University of New York Upstate Medical University and all participants provided written informed consent. Full details of the methods and issues involved in implementing the study have been published previously (Crawford et al., 2006). In summary, 103 pregnant women with asthma from the low-income, obstetric population of Syracuse were recruited into the study. Eligibility criteria included residence in the city of Syracuse or adjacent metropolitan location, proficiency in English or Spanish, and anticipated residence in the home for one year from the start of the study. All 103 homes were visited approximately three months after the infant's birth in order to obtain environmental samples, conduct inspections and interview occupants. Forty-three occupants were willing to have a second home visit. Second visits were intended to assess

changes in conditions over time and typically took place in a different season from the first.

2.2. Home inspection and occupant interviews

A trained field team conducted walk-through home inspections and occupant interviews. Home inspections were scheduled at the same time as the environmental sampling. Homes were inspected for housing type (detached house or part of a house versus apartment building). Each room was assessed for visible mold and musty odors. Water problems were defined by observation of water damage, standing water, plumbing leaks, damp stains or excessive condensation. We constructed a mold severity index by adding up all occurrences of visible mold, water problems, or musty odors. Discrete occurrences of mold-related problems were counted, and when different surfaces were affected such as walls, floors or ceilings, each surface contributed to the count. The mold severity index was categorized according to the number of mold occurrences: 0, 1–2 and ≥3. Cleanliness in the home was evaluated by the presence of unsealed garbage, food debris on surfaces, visible dirt, excessive clutter, evidence of pets (full litter boxes or animal droppings), and evidence of pests (cockroaches, rodents or droppings; each as yes or no). We noted discrete occurrences of these conditions and also constructed a composite variable called 'multiple housekeeping issues' by adding up individual episodes to indicate when homes had multiple occurrences of cleanliness-related conditions (more than one occurrence in more than one room).

Cockroaches and rodents were counted as present based upon either an occupant's positive report or an inspector's observation. Occupants were also interviewed about pets, home ownership, number of occupants and household smoking. Additional demographic information such as Medicaid status was collected through medical chart review. Use of humidifiers was recorded as was the presence of carpeting or large area rugs. We coded all dichotomous categorical variables as yes (if present) or no when appropriate.

2.3. Environmental samples

Air samples for viable fungi and bacteria were collected across all seasons. Samples were taken at the beginning and end of a 24-hour period using Andersen N6-type single-stage impactors which collect the sample onto culture media. Two indoor samples and one outdoor were taken on the first day of the home visit; one indoor sample and one outdoor sample were taken on the second day at the end of the 24-hour period. Indoor samples were taken in the main living area and outdoor samples were taken near the front of the home. The airflow rate through the impactor was checked at each use with a rotameter. Sample collection times were typically 3 min. In winter, when outdoor fungal levels were very low, collection times were extended to 6 min. Culture media consisted of 3% malt extract agar. In order to prevent rainwater or snow from entering the Andersen sampler, outdoor samples were taken in a protected location such as a front porch, side porch, deck or garage overhang. When there was no such structure, umbrellas were used to protect the sampler. To prevent freezing of agar plates, samplers and plates were kept indoors prior to sampling. Sampling was avoided during periods of extreme cold, sampling times were reduced or sampling was rescheduled if plates were observed to freeze. All samples were taken in sequential duplicate. Impactors were disinfected with 70% isopropyl alcohol prior to each use and between samples. Following collection, samples were kept cold and delivered on the same day to the laboratory.

Bioaerosol analysis methods were developed specifically for this project by SUNY College of Environmental Science and Forestry, Department of Construction Management and Wood Products Engineering. These methods included procedures to enhance detection of rare and slow-growing fungi (Catranis et al., 2006). In brief, air samples were collected onto sequential duplicate culture plates with one plate left untouched and used to determine the total colony count. The second plate was subsampled by removing 50 plugs of culture material from impaction sites and inoculating them onto fresh media. Without this subsampling, plates will tend to be dominated by common, fast-growing species. All plates were incubated at room temperature and colony forming units (CFUs) were counted twice; at 2–3 and 7–10 days after collection. Concentrations in CFU/m^3 were calculated according to the air volume sampled. Subsampled plates used an additional conversion factor to account for the fraction of plate evaluated. Cultures were transferred to slides for microscopic identification of genus and species following the methods of Wang (1990). Basidiomycete fungi were identified on the basis of clamp connections. Field blanks (n = 3) were taken at each home and handled just as sample plates except that no air was pumped through the impactor.

Real-time, direct readings were taken for temperature and carbon dioxide using a Langan Instruments L76 Indoor Air Quality Measurer (Langan Products Inc., San Francisco, CA). Readings were recorded at the start of the home visit and every 1 min thereafter for approximately 24 h. Instrument calibration was checked against a 1000 ppm carbon dioxide standard gas on a weekly basis. Relative humidity was recorded with a digital hygrometer (Fisher Scientific, NIST traceable calibration) as the average value over the duration of the home visit with the field team present (2–3 h).

Outdoor conditions including rain and snow cover were assessed on each of the 2-day home visits. Rain was classified as present if it occurred on either day. Snow cover was classified as present if it was noted on either day. Snow cover amounts were estimated in inches and the average between the two sampling days was used in analyses.

2.4. Data analysis

Total fungi was defined as the sum of all fungal CFUs detected regardless of taxon, and was reported as concentration per unit volume sampled. Mean fungal concentrations were calculated using all three indoor samples for each home. Based upon a minimum detection of 1 CFU per plate, detection limits were calculated as 12 CFU/m^3 for three-minute samples and 6 CFU/m^3 for six-minute samples. In the mean calculation, values below the detection limit were arbitrarily substituted with 2 CFU/m^3 for a 3 minute sample and 1 CFU/m^3 for a 6 minute sample, instead of substituting with LOD/2 or other more typical assignments.

Descriptive statistics included frequencies, means, standard deviations, medians, and percentiles. The ratio of indoor to outdoor fungal concentrations was used to assess the effect of snow cover. Ratios larger than 1 indicate greater indoor concentrations compared with outdoor.

Fungal distributions were highly skewed and log transformation did not normalize them. Therefore, non-parametric statistical procedures were used including Spearman's r for calculation of rank correlation, the Wilcoxon rank sum test for comparison of medians between two groups or Kruskal–Wallis one-way analysis of variance by ranks for comparison of three or more groups. We categorized indoor fungal levels as above or below the median concentration for analyses of the association between home conditions and fungal levels. This was done to facilitate an appropriate statistical analysis given the skewed concentrations and because there are no external standards or reference values for comparing airborne fungal concentrations. Only fungal genera that occurred at a frequency greater than 15% were used in further analyses. Bivariate (unadjusted) associations between each dichotomized taxon and each home condition (predictor) were examined with 2 × 2 contingency tables or by bivariate logistic regression. Odds ratios and two-tailed p-values from chi-squared tests were calculated. The predictors evaluated included season of home visit, indoor temperature, humidity, carbon dioxide level (an indicator of ventilation), visible mold, water problems, musty odors, outdoor concentrations of the corresponding fungus, use of a humidifier, home ownership, housekeeping issues, Medicaid insurance, greater than three children in the home

(an indicator of crowding), and presence of cat, of dog, of rodents, of cockroaches and of carpeting. A close relationship among visible mold, water problems and musty odors was anticipated as these conditions were often observed together within a house. As a preliminary step in evaluating these potentially overlapping factors, chi-square tests of independence were calculated for each pair of variables. Smaller p-values suggest the variables are closely related. A similar calculation was also made for the overlapping variables multiple housekeeping issues and the presence of cockroaches.

We used multivariable logistic regression to assess the importance of predictors when considered together. For each fungal genus, predictors were identified from the bivariate analyses. Explanatory variables that were associated with the outcome (each dichotomized taxon) with a p-value of approximately 0.1 or less in bivariate analyses were considered as candidates for inclusion in multivariable logistic regression. When candidate variables were strongly associated (overlapping), only the one with the largest magnitude bivariate odds ratio was included in the multivariable model. The final models were developed using both stepwise and backward logistic regression procedures. The final models included variables significant at $\alpha < 0.05$. We evaluated the adequacy of model fit by calculating the Hosmer–Lemeshow goodness of fit statistic; p-values >0.05 for this statistic indicate adequate fit (Hosmer et al., 1991). The generalized coefficient of determination (Cox and Snell R^2) was also reviewed. Larger R^2 values suggest better models. The concordant index c (equivalent to the area under the receiver-operator curve (ROC)) was also examined to assess model adequacy. Larger values indicate stronger associations between the observed and predicted values. In terms of correct prediction of models, values between 0.6 and 0.7 are considered a poor fit, those between 0.7 and 0.8 are acceptable, values between 0.8 and 0.9 suggest excellent discrimination and values above 0.9 are outstanding (Hosmer and Lemeshow, 2000). JMP 10.0 and SAS 9.3 (both SAS Institute Cary, NC) were used for statistical analyses.

3. Results

3.1. Home conditions

In this low-income urban population, the majority of participants had Medicaid for health insurance, rented an apartment or house and had smokers in their home (Table 1). Moisture indicators were observed frequently with over 70% of homes showing some signs of water problems during the home inspection. Musty odors (37%) and visible mold (24%) were less common. Most homes were reasonably clean, however 24% were found with housekeeping issues such as excessive clutter, uncontained garbage, evidence of pets or pests, visible dirt, and odors. One quarter of homes had cockroaches and 30%, dogs. Indoor temperatures ranged from 19 to 34 °C (67 to 93 °F). Home temperatures were warm throughout the year averaging 28 °C (82 °F) in summer and 25 °C (77 °F) in each of the fall, spring and winter seasons. Indoor relative humidity varied according to season with average levels of 53% in summer and 35% in winter. Moisture and mold-related observations overlapped considerably within homes (Fig. 1). All 38 homes with musty odors had water problems and 23/25 homes with visible mold also had water problems. Fourteen of 25 homes with mold also had musty odors.

3.2. Fungal prevalence, distributions and correlations

Detectable fungi were present in all homes, with over 170 taxa (genus or species) identified. The most frequently occurring taxa were hyaline unknowns, *Penicillium*, *Cladosporium*, yeasts, *Aspergillus*, dark unknowns, basidiomycetes, *Acrodontium* and *Alternaria*. Hyaline "unknowns", non-sporulating fungi with light-colored hyphae, were detected most often. They were present in 86% of homes indoors and 84% outdoors (Fig. 2). A surprising result was *Acrodontium*, a genus that is rarely reported in indoor air, and was found here in 16% of study homes. Detection of *Acrodontium* was probably due to the analysis method that allowed recovery of slow-growing fungi. Other noteworthy fungi were identified through this method and have been reported previously (Fernando et al., 2005). However, only *Acrodontium* was present at a high enough frequency to allow further assessment of its relationship with home conditions. *Aspergillus*, *Penicillium*, yeasts and bacteria were detected more frequently indoors compared with outdoors (Fig. 2) and this was true in both warm and cold seasons (data not shown).

For these predominant fungi, we also examined concentration distributions including means, medians, and percentiles (Fig. 3). Overall, taxa that were detected most frequently were also found at the highest concentrations. For example, hyaline unknowns were most prevalent and also occurred at the highest levels, with a median concentration of 286 CFU/m^3 outdoor and 159 CFU/m^3 indoor. *Aspergillus* and *Penicillium* were detected more frequently indoors than outdoors, and also had higher indoor concentrations compared with outdoor. In contrast,

Table 1
Home characteristics of the cohort.

Characteristic	Visit 1 (n = 103) %	
Demographics		
Rent house or apartment	85	
Medicaid insurance	82	
>3 children in home	28	
Moisture-related		
Water problem	71	
Musty odor	37	
Visible mold	24	
Mold severity index[a]		
0	27	
1–2	35	
≥3	38	
Humidifier in use	19	
Home conditions, cleanliness		
Household smoking	68	
Carpets in main living room	65	
Unsealed garbage	45	
Food matter on surfaces	33	
Multiple housekeeping issues	26	
Pets and pests		
Dog in home	30	
Cat in home	23	
Observed or reported cockroaches	23	
Observed or reported rodents	17	
Mean indoor temperature, degrees F (standard deviation)	78.1	(4.6)
Mean indoor relative humidity, % (standard deviation)	43.2	(12.8)
Mean carbon dioxide level, parts per million (standard deviation)	777	(327)

[a] Total number of observations of visible water, water damage, musty odor and visible mold in the home.

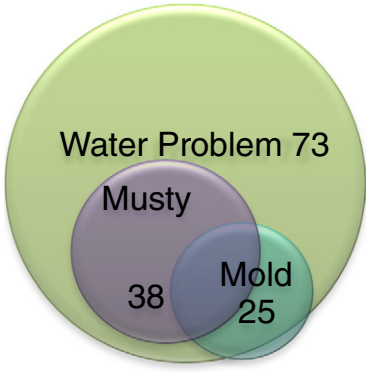

Fig. 1. Number of homes with overlapping moisture-related problems.

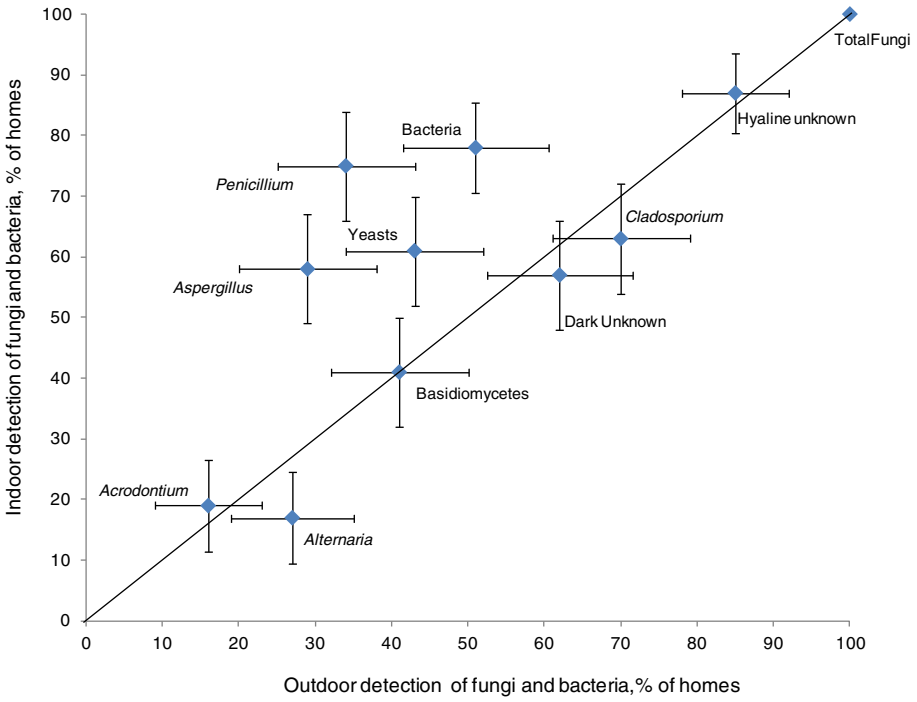

Fig. 2. Relationship between indoor and outdoor frequency of detection of fungi and bacteria. Points above the 1:1 line indicate the taxa were detected more frequently indoor than outdoor. Error bars represent the 95% confidence interval of the frequencies.

Alternaria and *Cladosporium* had higher concentrations outdoor compared with indoor.

Over a year's time, both indoor and outdoor total fungal concentrations showed substantial monthly variation with values highest in August and lowest in February and March (Fig. 4). Clearly, indoor concentrations track outdoor concentrations closely for total fungi. The peaks correspond to warm weather where temperatures stimulate fungal growth and vegetation is abundant for colonization. The strength of the relationship between indoor and outdoor concentrations was assessed by correlation analyses for all fungal types. Most genera had moderately to highly correlated indoor to outdoor levels (Spearman's $r > 0.5$) and all correlations were significant. Indoor and outdoor levels were most strongly correlated for *Acrodontium* ($r = 0.83$), *Cladosporium* ($r = 0.79$), and *Alternaria* ($r = 0.74$). The weakest correlations were seen between indoor and outdoor levels of *Penicillium* ($r = 0.23$), yeasts ($r = 0.31$) and *Aspergillus* ($r = 0.33$).

Most of the fungi showed considerable variation in seasonal concentration (Fig. 5). A variety of patterns were seen with the highest concentrations often occurring in summer or fall. In the examples shown, *Cladosporium* and hyaline unknowns show a pattern of higher outdoor levels compared with indoor along with summer and fall maximum levels. The proliferation of plant material in the growing season accounts for the peaks in *Cladosporium*, a phylloplane genus. In contrast, *Penicillium* and *Aspergillus* show a pattern of higher indoor levels

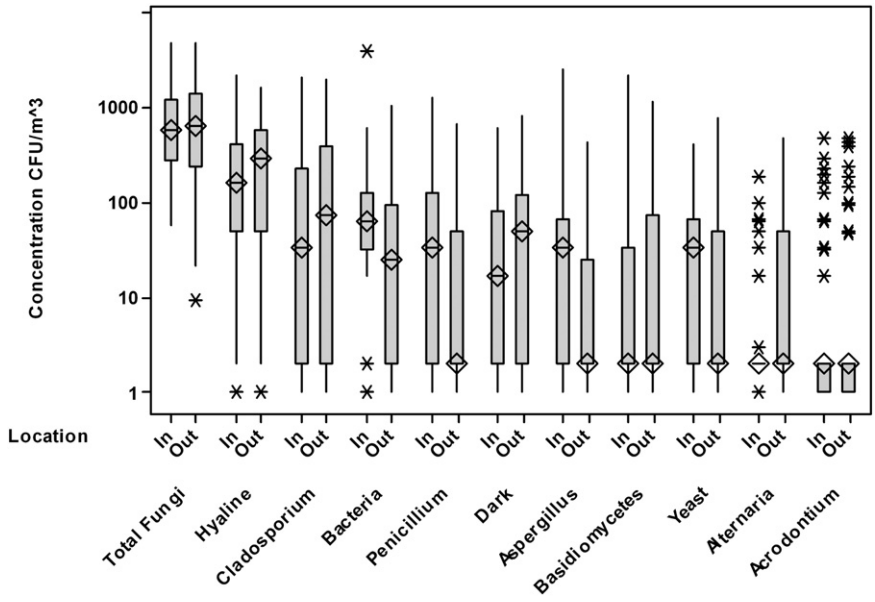

Fig. 3. Concentrations of indoor and outdoor fungi and bacteria n = 103 study homes, visit 1. Medians are indicated by a diamond, outliers are 1.5 times the interquartile range and are indicated with a star (*).

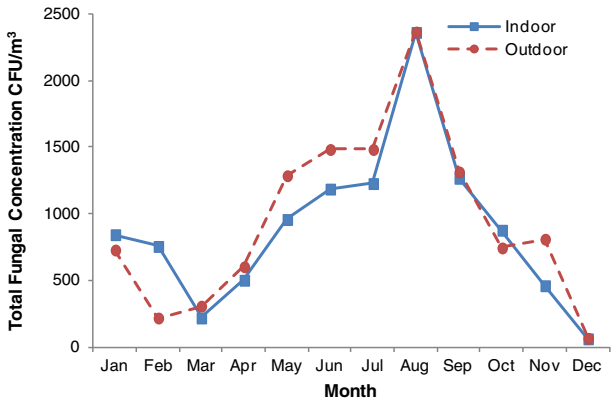

Fig. 4. Indoor and outdoor mean monthly total fungal concentrations, Syracuse, NY 2002. N = 93 homes, visit 1.

compared with outdoors across the seasons. The seasonal variation for all fungi was examined using the non-parametric Kruskal–Wallis test, with separate one-way tests for indoor and outdoor cases (data not shown). Differences in median concentrations across seasons were significant ($p < 0.05$) for most fungi, indoors and outdoors with the exceptions of indoor *Aspergillus*, basidiomycetes and bacteria (both indoor and outdoor).

3.3. Snow cover and indoor to outdoor ratios

The data collected in this project across two winters provided the opportunity to examine the effect of snow cover on indoor and outdoor fungal levels. Syracuse, NY is noteworthy as the snowiest major city in the contiguous United States with an average yearly snowfall of 119 in. (Northeast Regional Climate Center, 2011). At the first home visit, there were 25 homes with snow cover and 76 homes without (values were missing for two homes). Of the homes with snow, the depth ranged from 0.75 to 6 in.. A time series of indoor to outdoor (I/O) ratios for total fungi (Fig. 6) shows a pattern of increased I/O ratios with snow cover present compared to days without snow cover. The highest ratios coincide with days when snow covers the ground. Over the entire series, the overall median I/O ratio was 0.9 with a range of 0.14 (June) to 29 (February). Homes with snow on the ground had a median I/O ratio of 3 while those without snow cover had a median ratio of 0.78. These ratios were compared with the Wilcoxon rank sum test and the difference was highly significant ($p < 0.001$). We analyzed fungal concentrations during the cold season only and found that snow cover depressed outdoor concentrations more than indoor (Fig. 7). For 47 homes visited during the cold season, the median outdoor total fungal concentration was 548 CFU/m^3 with no snow present and 83 CFU/m^3 with snow cover (Wilcoxon rank sum test $p < 0.001$). At the same time, the median indoor total fungal concentration was 427 CFU/m^3 with no snow and 238 CFU/m^3 with snow cover present (Wilcoxon rank sum test $p = 0.14$).

We assessed the effect of snow cover on I/O total fungal ratios for the subset of homes with a second home visit. The results were nearly identical to those of the first visit. At visit 2, 11 homes had snow cover, with a median total fungi I/O ratio of 2.9 Thirty homes had no snow cover and a significantly lower ($p < 0.0001$) median I/O ratio of 0.83. The relationship between snow cover and indoor to outdoor ratios for other fungal types was also examined. For most fungal types, I/O ratios tended to be higher with snow cover present compared with no snow, but differences were not significant. This may be because low detection during winter effectively reduced the sample sizes for comparison.

We also evaluated the effect of rain on indoor to outdoor ratios for total fungi. This analysis was restricted to 54 homes visited in warm weather (April–September) when there was no snow cover. During

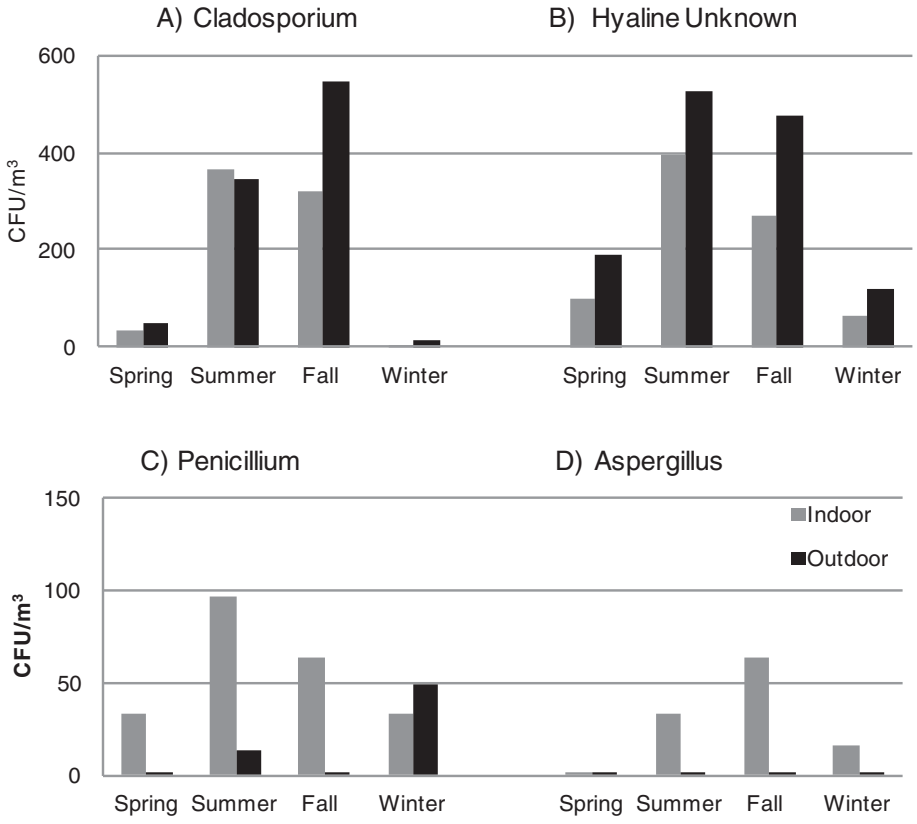

Fig. 5. Seasonal indoor and outdoor median concentrations in CFU/m^3 for various fungi, visit 1. Separate, indoor and outdoor one-way Kruskal–Wallis tests of differences in medians across seasons were significant at $\alpha < 0.05$ for the fungal types shown above, except for indoor *Aspergillus* ($p = 0.06$).

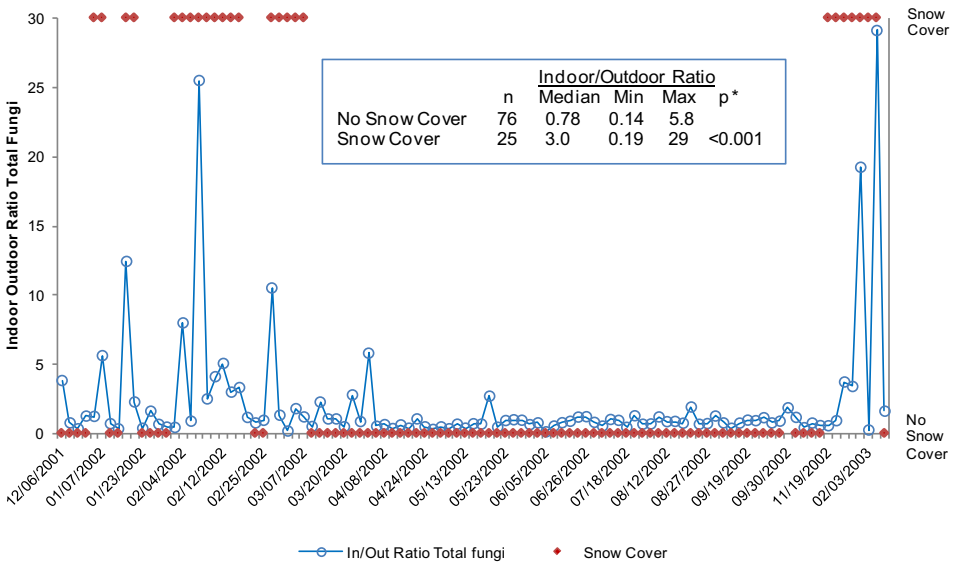

Fig. 6. Indoor/outdoor ratio of total fungi and snow cover time series, visit 1. *p-value is for the significance of the Wilcoxon test of the difference between medians.

rain, I/O ratios were slightly lower but not significantly different from non-rain days. For 31 homes visited when there was no rain, the median I/O ratio was 0.82 (minimum 0.36, maximum 5.8). Of 23 homes visited during rain, the median I/O ratio was 0.67 (minimum 0.14, maximum 2.8). These differences were not significant (Wilcoxon rank sum test, $p = 0.16$).

3.4. Predictors of indoor fungi

Contingency tables and logistic regression were used to evaluate bivariate relationships between individual predictors (independent variables) and high indoor fungal levels (dependent variables) for the predominant indoor fungi (Fig. 8). The presence of cockroaches was positively related (e.g. odds ratio >1) to elevated levels of all fungal types except yeasts. However, cockroaches only showed a significant relationship ($p < 0.05$) with total fungi (OR 3.05 95% CI 1.14–8.18), *Aspergillus* (OR 3.97 95% CI 1.42–11.1) and *Penicillium* (OR 5.58 95% CI 1.89–16.5). Homes with musty odors were also positively related to high levels of most fungal types with total fungi (OR 2.71 95% CI 1.18–6.22) and *Penicillium* (OR 3.47 95% CI 1.49–8.09) statistically significant. Observation of visible mold in the home predicted significantly higher levels of *Penicillium* (OR 3.51 95% CI 1.31–9.36) and bacteria (OR 3.33 95% CI 1.35–8.88). Although water problems were present frequently in homes and odds ratios were above one for several fungal types, they were not significantly associated with high levels of any fungi. Home ownership, a socioeconomic indicator, predicted lower levels of *Cladosporium* (OR 0.15 95% CI 0.03–0.69) and total fungi (OR 0.3 95% CI 0.09–1.03). Having Medicaid for insurance predicted significantly elevated levels of *Penicillium* (OR 3.39 95% CI 1.12–10.3).

Across all fungal types, more consistent results were seen for the effects of indoor humidity and temperature (Fig. 8). Higher indoor relative humidity was positively related to high levels of all fungal types and most results were significant. Higher indoor temperatures also were positive predictors for high levels of fungi with the majority of results statistically significant. Higher outdoor concentrations were consistent and significant predictors of high corresponding indoor fungal levels for nearly all taxa, with the exception of *Penicillium*. Air samples collected during the cold season were consistently associated with lower fungal levels compared with warm season samples. Total fungi, *Acrodontium*, *Alternaria*, *Penicillium*, *Cladosporium* and yeast concentrations were significantly lower in cold seasons. Homes with multiple housekeeping issues (e.g. multiple occurrences of unsealed garbage, food debris, visible dirt, clutter, evidence of pests or pets) had a significantly greater odds of elevated total fungi (OR 2.47 95% CI 0.99–6.19).

Other predictors were assessed but were not significant at $p < 0.05$ although some had marginally significant ($p < 0.1$) relationships with

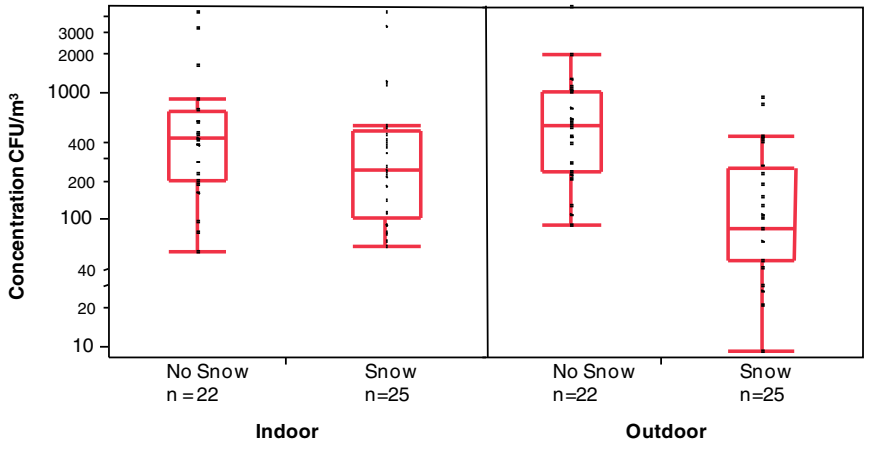

Fig. 7. Effect of snow cover on indoor and outdoor total fungal concentration during the cold season (Oct.–Mar) only. N = 47 homes, visit 1. The Wilcoxon test of the difference between no snow and snow outdoor median concentrations was significant ($p < 0.001$).

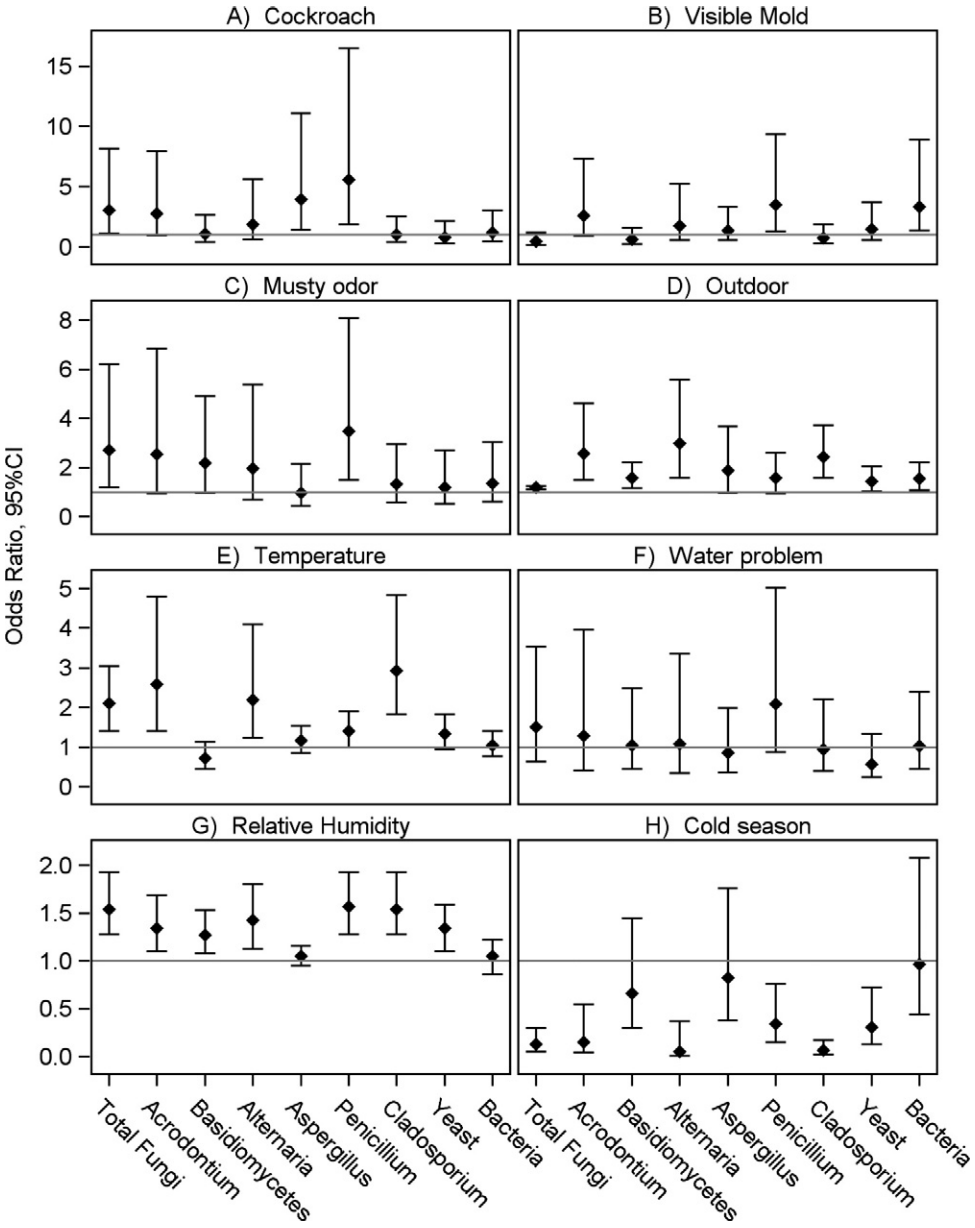

Fig. 8. Association between high indoor airborne fungal levels and individual predictors. Unadjusted odds ratios with 95% confidence intervals (n = 103 visit 1). High levels are defined as above the median concentration for total fungi, *Aspergillus*, *Penicillium*, *Cladosporium* and yeasts and above detection for *Acrodontium*, Basidiomycetes and *Alternaria*. For A) Cockroach, B) visible mold, C) musty odor, and F) water problem, odds ratios compare presence versus absence of the characteristic. D) Outdoor refers to the concentration of the corresponding fungus or bacteria with each 50 CFU increase. E) Temperature refers to an increase of 5 °F. G) Relative humidity refers to an increase of 5%. H) Cold season (November–April) versus warm season (May–October).

some taxa. These predictors include the mold severity index, presence of cats, dogs, humidifiers in the home, and carpeting, number of children in the home and carbon dioxide levels.

Multivariable logistic regression models were used to assess the independent effects of predictor variables (Table 2). Total fungi had many significant predictors in the bivariate case including outdoor total fungi, musty odors, indoor relative humidity, indoor temperature, cockroaches, visible mold, home ownership, housekeeping issues and season of visit. However, the multivariable model showed that outdoor total fungi and musty odors were the only significant predictor variables at the 0.05 level. For most fungal types except *Penicillium* and yeasts, outdoor concentrations remained significant predictors of high fungal levels. Although season of home visit was significant in many of the bivariate analyses, it was an independent predictor only for *Cladosporium* and *Alternaria*. Samples collected during warm seasons had four times the odds of elevated *Cladosporium* (OR 4.30 95% CI 1.16–17.8) and more than ten times the odds of elevated *Alternaria* (OR 10.8 95% CI 1.87–205) compared with samples collected during cold seasons. Temperature and humidity were not consistently important independent predictors, appearing in some, but not all models. However for yeasts, indoor relative humidity was the only independent predictor of high airborne concentrations. Increasing indoor temperature predicted lower levels of basidiomycetes (OR 0.43 95% CI 0.21–0.78).

Cockroaches remained significant predictors of high levels of *Aspergillus* (OR 3.97 95% CI 1.42–11.1) and *Penicillium* (5.58 95% CI 1.89–16.5) in the final models. Visible mold was also a significant independent predictor of high levels of *Penicillium* (OR 3.51 95% CI 1.31–9.36) as well as for bacteria (OR 3.79 95% CI 1.41–11.2). Musty odor was a significant predictor of high basidiomycete concentrations (OR 4.26 95% CI 1.37–14.7) whereas the presence of a dog predicted lower concentrations (OR 0.19 95% CI 0.05–0.64). And humidifiers in use predicted lower levels of *Acrodontium*.

Table 2
Predictors of indoor airborne fungi and bacteria: Logistic regression models with adjusted odds ratios (AOR) and 95% confidence intervals (CI) (N = 103 visit 1 or as noted).

Dependent variable[a]	Predictor variables	AOR	(95% CI)	Model fit statistics[b]
Total Fungi	Outdoor total fungi (per 50 CFU/m^3)	1.18	(1.11–1.27)	$R^2 = 0.46$
	Musty, moldy odor (yes/no)	3.48	(1.13–11.6)	ROC = 0.90
				H–L p = 0.14
Aspergillus	Outdoor *Aspergillus* (per 50 CFU/m^3)	2.00	(1.13–4.54)	$R^2 = 0.13$
	Cockroaches (yes/no)	4.36	(1.60–13.4)	ROC = 0.72
				H–L p = 0.10
Penicillium	Indoor relative humidity (per 5%)	1.34	(1.11–1.64)	$R^2 = 0.27$
	Cockroaches (yes/no)	3.66	(1.16–13.1)	ROC = 0.80
	Visible mold (yes/no)	4.11	(1.37–14.0)	H–L p = 0.18
	Insurance (Medicaid/private)	3.72	(1.13–14.5)	
Cladosporium	Outdoor *Cladosporium* (per 50 CFU/m^3)	2.34	(1.61–3.90)	$R^2 = 0.56$
	Warm season (reference cold season)	4.30	(1.16–17.8)	ROC = 0.95
				H–L p = 0.95
Yeast	Indoor relative humidity (per 5%)	1.31	(1.11–1.58)	$R^2 = 0.10$
				ROC = 0.68
				H–L p = 0.78
Bacteria	Outdoor bacteria (per 50 CFU/m^3)	1.60	(1.18–2.45)	$R^2 = 0.16$
	Visible mold (yes/no)	3.79	(1.41–11.2)	ROC = 0.80
				H–L p = 0.03
Acrodontium	Outdoor *Acrodontium* (per 50 CFU/m^3)	4.11	(2.14–9.90)	$R^2 = 0.36$
(N = 100)	Indoor temperature (per 5° F)	3.49	(1.54–10.0)	ROC = 0.90
	Humidifier (yes/no)	0.003	(<0.001–0.27)	H–L p = 0.3
Alternaria	Outdoor *Alternaria* (per 50 CFU/m^3)	2.26	(1.36–4.49)	$R^2 = 0.26$
	Warm season (reference cold season)	10.8	(1.87–205)	ROC = 0.87
				H–L p = 0.17
Basidiomycetes	Outdoor basidiomycetes (per 50 CFU/m^3)	1.62	(1.20–2.44)	$R^2 = 0.33$
	Indoor relative humidity (per 5%)	1.35	(1.10–1.70)	ROC = 0.82
	Indoor temperature (per 5° F)	0.43	(0.21–0.78)	H–L p = 0.18
	Dog (yes/no)	0.19	(0.05–0.64)	
	Musty, moldy odor (yes/no)	4.26	(1.37–14.7)	

[a] Total fungi, *Aspergillus*, *Penicillium*, *Cladosporium*, yeasts and Bacteria were dichotomized at the median of their distributions. *Acrodontium*, *Alternaria* and Basidiomycetes were dichotomized as above detectable levels vs not detected. All of the above variables are significant at $p < 0.05$.
[b] R^2 refers to the generalized coefficient of determination (Cox & Snell R^2), ROC is the area under the receiver–operator curve, H–L p-value is given for the Hosmer–Lemeshow goodness of fit test.

4. Discussion

In this study we assessed the types and concentration of fungi in the indoor air of homes of infants in low-income, urban areas of Syracuse, NY. *Cladosporium*, *Penicillium*, non-sporulating fungi and *Aspergillus* are among the most common culturable fungi found in indoor and outdoor air across the US (Shelton et al., 2002). These fungi also occurred frequently in our study. *Acrodontium*, a genus that is rarely reported in indoor air samples, was found likely due to the analysis protocol that facilitated recovery of slow-growing fungi.

In contrast with other studies, we report a high frequency of basidiomycetes, present in 41% of homes. Most surveys of culturable airborne fungi do not mention basidiomycetes (Chew et al., 2003; Garrett et al., 1998; Ren et al., 2001; Shelton et al., 2002). Using non-viable air sampling methods such as spore traps, basidiospores have been reported as abundant in residential samples (Osborne et al., 2006) and in schools in widely differing geographic locations in the US (Levetin et al., 1995). An epidemiology study in the UK that used culture-based methods plus additional testing reported basidiomycetes in the top 5 most abundant fungal types (Strachan et al., 1990). Because of the difficulty in identifying basidiomycetes in culture, they are likely to be reported as non-sporulating fungi or unknowns. Even with expertise to identify basidiomycetes, our study still found that 89% of homes had non-sporulating fungi that we reported as hyaline unknowns. A subsequent investigation to further identify the hyaline unknowns from our study revealed that most were probably basidiomycetes (Anagnost et al., 2006). We note that basidiomycetes may be prevalent yet under-recognized in indoor air when culture-based sampling methods are employed. Basidiospores are produced by mushrooms and similar fungi and are commonly found outdoors. When detected indoors, they typically come directly from an outdoor source, however dry rot of wooden structures can be an indoor source of basidiospores. Like many fungi, basidiomycetes are associated with allergic responses (Horner et al., 1995).

The presence of snow cover suppressed outdoor fungal concentrations and indoor to outdoor total fungal concentration ratios were increased. With snow cover present, indoor concentrations were typically three times higher than outdoor (median I/O ratio of 3), but could be nearly 30 times higher (maximum I/O ratio of 29, Fig. 6). This is of practical importance to indoor air investigators because I/O concentration ratios are often used for interpretation of air sampling results. Ratios less than 1 suggest that outdoor fungal sources are dominant while ratios greater than 1 may suggest that indoor fungal contamination is present. A study of over 1700 buildings throughout the US established that 85% of the buildings had I/O ratios of 1 or lower for total fungi (Shelton et al., 2002). Investigators should be aware that snow cover can temporarily increase I/O ratios and interpretation should be made with caution. This subject is not well documented in the literature and available reports do not characterize temperate climates with episodic snowfall. Reponen showed that in the subarctic climate of Finland, where the ground is frozen and snow-covered during winter, I/O fungal concentration ratios in homes averaged 1.4 in the winter and 0.4 in the summer while I/O ratios for bacteria averaged 27.5 in winter and 4.5 in summer (Reponen et al., 1992). That report recommended taking indoor samples in subarctic climates when the ground is continuously frozen and snow-covered in order to detect abnormal indoor sources. We note rather the opposite; with thaws and periodic snowfall, I/O ratios may be sporadically increased making it difficult to interpret these ratios and to identify truly abnormal indoor sources.

We also examined the relationship between home characteristics and indoor airborne fungal exposure. We found that home characteristics predicted high indoor fungal exposure specific to taxon. In multivariable analyses, fungal levels were higher in homes with visible mold,

musty odors, and cockroaches but not for all genera studied (Table 2). Similarly, home conditions such as higher temperatures and humidity predicted high concentrations of some fungi but not others. One of the most consistent predictors of high indoor fungal levels across all genera was corresponding outdoor fungal concentrations. This underscores the need to use multivariable analyses and account for outdoor concentrations in studies of this kind.

Our home inspection focused on conditions indicative of fungal growth as well as conditions capable of supporting fungal growth. When visible mold is present in homes, there is an obvious potential for fungal exposure. Visible mold was found in one-quarter of homes, but in the final models it was associated only with high levels of *Penicillium* and bacteria. Musty odors may be an important indicator of indoor fungal growth and potential exposure, but they were independent, significant predictors only for total fungi and basidiomycetes. Because moisture is a fundamental requirement for fungal growth, we sought to identify moisture and water-related problems such as water damaged material, standing water, leaks or damp stains. Water problems were observed in the majority of homes but were not associated with any fungus in multivariable analysis. One possible reason for this lack of association is that we did not specify the size of a water problem, such that trivial water problems may have been counted along with more substantial ones thus obscuring any effect. We also did not find an association between the mold severity index and high fungal levels. The mold severity index summed up all occurrences of moisture, mold and musty odors in the home in order to capture the extent and severity of mold-related problems. Other studies have characterized the intensity of mold damage/exposure with qualitative categorization systems and some have found dose-dependent relationships between intensity and health outcomes (Cho et al., 2006; Haverinen et al., 2001; Mommers et al., 2005) or fungal levels (Dharmage et al., 1999) whereas others have not (Reponen et al., 2010).

Several studies have found a positive relationship between moisture and mold in the home and indoor fungal concentrations while others have not. Among studies that report an association, the results are generally similar to ours in that moisture and mold are related to higher levels of some, but not all fungi. For instance, in seven major US cities, inner-city homes of asthmatic children that had water leaks or moisture in the child's bedroom were associated with higher levels of total fungi and *Cladosporium* but not *Aspergillus*, *Penicillium* or *Alternaria* (O'Connor et al., 2004). Signs of dampness in Parisian babies' homes were related to total fungi and *Aspergillus* but not *Cladosporium*, *Penicillium* or *Alternaria* (Dassonville et al., 2008). In Australian homes, visibly moldy surfaces were related to total viable fungi (Dharmage et al., 1999). Another Australian study found that visible mold growth or condensation was associated with *Cladosporium* but not total fungal concentrations (Garrett et al., 1998). In Connecticut homes, only basement water sources were related to higher fungal concentrations (Mahooti-Brooks et al., 2004). Studies that have not found a relationship between fungal levels and home conditions include a report of Swedish children's homes where indoor concentrations of culturable fungi (including *Aspergillus*, *Cladosporium*, *Penicillium* and total fungi) were not associated with signs of dampness or moldy odor (Holme et al., 2010). A Boston-area study found that occupant's reports of mold, mildew and water damage were not related to total fungal levels (Chew et al., 2003). In a study of one thousand children's homes in the northeastern US, reported mold growth and damp patches did not reliably predict fungal concentrations in indoor air (Ren et al., 2001). Because there are no standard methods for bioaerosol sampling or for home inspection, these are probable reasons for the differences in findings. Geographic or climatic differences and lack of control for outdoor concentrations may also account for conflicting findings.

Different methods of data analysis also contribute to disparate findings between studies. We characterized high levels of indoor fungi as above the median concentration to facilitate an appropriate statistical analysis and because there are no standards for comparing airborne fungal concentrations. A variety of approaches have been tried by other studies with some authors using median or percentile concentrations as a cut point (Chew et al., 2003; Dassonville et al., 2008; Jones et al., 2011; Stark et al., 2005). Others authors have evaluated fungal concentrations as continuous variables (Dassonville et al., 2008; Dharmage et al., 1999). The difference between indoor and outdoor concentrations has been suggested as a method to also account for outdoor levels (O'Connor et al., 2004). Another report describes a qualitative procedure whereby indoor and outdoor air genera were compared subjectively by an expert team resulting in an assigned mold index rating (Holme et al., 2010). The variety of evaluation methods highlights the lack of consistency in data analyses that undoubtedly contribute to different findings between studies.

In our study, the presence of cockroaches predicted significantly higher *Aspergillus* and *Penicillium* levels. Cockroaches require water for survival, and they thrive in moist, warm environments. Their presence may indicate chronic moisture in the home which would be supportive of fungal growth. However, for both *Aspergillus* and *Penicillium*, water problems themselves did not predict high fungal levels. The presence of cockroaches was significantly related to water problems (Pearson's chi square $p = 0.04$, data not shown). Thus cockroaches may serve as a good proxy for water problems, particularly those that are chronic. Chronic moisture problems and the presence of cockroaches likely serve as indicators of underlying disrepair of the building. Housing disrepair and deterioration such as leaking pipes, water damage, holes in walls and housing code violations have been linked to cockroach infestation and cockroach allergen in other studies (Peters et al., 2007; Rauh et al., 2002; Rosenfeld et al., 2011). An inner-city asthma study also reported a positive association between cockroaches and *Aspergillus* concentrations (O'Connor et al., 2004). Most other studies follow higher socioeconomic populations where cockroaches are not prevalent and thus are not reported. Cockroaches are a public health concern because they produce allergens that can exacerbate asthma and allergies and because they can carry pathogens that cause disease. Our results suggest that in addition to the potential for allergen and pathogen exposures, the presence of cockroaches may also indicate fungal exposures. One of the main tenets of cockroach control is removal of moisture and water sources. Therefore cockroach control may also be beneficial in reducing fungal exposures.

Of all the factors considered, outdoor fungal concentrations were the most consistent predictors of indoor levels. Outdoor fungal levels vary widely as they are affected by temperature, humidity, season, wind, and precipitation. Geographic location and the surrounding agricultural landscape are also important determinants of outdoor concentrations. Like ours, many studies demonstrate correlation between indoor and outdoor fungal levels (Chew et al., 2003; Dassonville et al., 2008; Garrett et al., 1998; Li et al., 1995; Li and Kendrick, 1995; O'Connor et al., 2004; Shelton et al., 2002). The close relationship between indoor and outdoor total fungal levels (illustrated in Fig. 4) highlights the need to account for outdoor levels in predictive models.

In the final model for *Acrodontium*, the presence of humidifiers was related to a lower odds of detecting *Acrodontium*. Homes where humidifiers were in use tended to have slightly lower relative humidity (41.4%) compared to homes without humidifiers (44.1%). Although higher humidity might be expected to result from humidifier use, humidifiers were deployed more frequently in the cold season than warm season (data not shown) most likely to raise low humidity levels during winter. It is unclear why the presence of dogs was associated with lower levels of basidiomycetes. While this could be a chance finding, the presence of cats also showed an inverse association with basidiomycetes in bivariate analysis (data not shown). Other studies tend to show that pets are related to higher indoor fungal concentrations presumably from tracking in fungal-contaminated materials from outdoors or from moisture related to cat litter boxes (Chew et al., 2003; Dharmage et al., 1999; O'Connor et al., 2004; Ren et al., 2001). In our study, perhaps the presence of pets is related to more frequent opening

of exterior doors. If indoor basidiomycete sources are present, opening doors will tend to dilute basidiomycete concentrations. This subject requires further study.

In our previous study, we found that high levels of indoor airborne *Penicillium* were associated with episodes of wheeze in infants living in low income areas of Syracuse (Rosenbaum et al., 2010). Here we have determined that the underlying home conditions giving rise to elevated levels of *Penicillium* include the presence of visible mold, cockroaches, increasing relative humidity, and Medicaid for insurance. Together, our findings suggest that controlling these conditions may be important for preventing infant wheeze. The majority of participants rented their homes or apartments and had Medicaid, an indicator of poverty. Thus, they may lack the capacity or resources for modifying their home conditions. Our report supports the need for public health intervention to provide healthy homes in low-income areas.

The use of short term air samples to characterize fungal exposures is a limitation of this study. Longer duration air samples are not practical as this will cause overgrown, unreadable plates. Although samples were only 3 to 6 min in duration, we attempted to improve their reliability by taking three samples over a 24 hour period and using the mean value. Nonetheless, because of high variability in fungal levels, exposures may not be represented very well by short-term air samples (Heinrich et al., 2003). A further limitation is introduced by the varying sample times. Six-minute sample times were used occasionally to enhance collection of outdoor fungal spores when concentrations were low. However, with longer sampling times, the collection of microorganisms can be reduced from dehydration and stress on the organism and from desiccation of the agar which leads to particle bounce and loss (Juozaitis et al., 1994; Mainelis and Tabayoyong, 2010; Xu et al., 2013). Multivariable model fit statistics indicate that some models have poor fit or predictive ability or both. R^2 values for logistic regression are typically much lower than those for linear regression, however both yeast and bacteria had very low values (0.1 and 0.16 respectively). In addition the H–L goodness of fit statistic for bacteria had a p-value of 0.03 indicating that the model does not fit the data well. The ROC value (concordant index) of 0.68 for yeast indicates poor prediction for the model. Together these results suggest that important predictors may not have been measured in these cases. More robust results might also result from a larger sample size.

4.1. Conclusions

In conclusion, the dominant fungal types in low-income homes of infants in and around Syracuse, NY included an uncommon genus *Acrodontium* as well as the commonly reported genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*. Basidiomycete fungi occurred frequently and may be under-reported in studies where culture-based sampling methods are used. When snow covers the ground, outdoor total fungal levels are depressed and indoor/outdoor concentration ratios are increased. Indoor air investigators should interpret these ratios cautiously. Home characteristics such as visible mold, musty odors, cockroaches and relative humidity were associated with high levels of some fungi, but not others. Control of these conditions may be important for reducing fungal exposures and preventing adverse health effects. Cockroach control that focuses on removal of moisture sources may have an added benefit of reducing fungal exposures.

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