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Polychlorinated Biphenyls (PCBs): Impact on Bat Activity and Foraging Behaviour along the Upper Hudson River, New York

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Abstract

Sediments of the upper Hudson River, New York, contain polychlorinated biphenyls (PCBs). Consequently, elevated levels of PCBs have been found in the tissues of bats and their insect prey along this region. However, it is not clear whether bat activity and foraging behaviour have been affected. To assess possible effects of PCBs on bat activity and foraging behaviour, we measured *Myotis lucifugus* (little brown bat; LeConte, 1831) and *Lasiurus cinereus* (hoary bat; Beauvois, 1796) activity along the upper Hudson River, as well as abundance of insect prey at the same locations. We also measured foraging duration and distances travelled by radio-tagged *M. lucifugus*. We found that bat activity and insect abundance did not differ with PCB concentration. We did, however, find that foraging behaviour along the Hudson River differed from a control site. Specifically, *M. lucifugus* foraging along PCB contaminated areas of the Hudson River traveled shorter distances from their roosts, and spent less time foraging than bats at an uncontaminated site. Our results show that while bats roost and forage in areas historically exposed to PCBs, this exposure has not adversely affected bat activity, foraging behaviour, or abundance of insect prey.

Keywords: PCBs, Hudson River, *Myotis lucifugus*, little brown bat, *Lasiurus cinereus*, hoary bat, bat activity

Introduction

For over 40 years, polychlorinated biphenyls (PCBs) were used extensively as insulators and fire-retardants in transformers and other industrial applications (Ross 2004). In 1976, the U.S. Congress banned PCB production (United States Environmental Protection Agency [USEPA] 1979; Ross 2004) in the wake of environmental (Jensen 1972) and laboratory studies indicating that PCBs have health effects on animals (e.g., Nagasaki et al. 1972; Loose et al. 1978), even though the risk of environmental PCB exposure to humans may be low (Ross 2004). However, PCBs persist in the environment, raising questions about their potential impact on wildlife exposed to these compounds across generations.

From the late 1940s to 1977, two General Electric Company (GE) manufacturing plants, one in Hudson Falls, NY (43°18'N, 73°35'W), the other in Fort Edward, NY (43°16'N, 73°35'W) discharged PCBs into the upper Hudson River (USEPA 2000). In 1984, the upper Hudson River from Ft. Edward to Troy, New York, was designated by the U. S. Environmental Protection Agency (USEPA) as a Superfund Site, indicating that it was a hazardous waste site that could have impacts on the local ecosystem and people (USEPA 2000). In 2002, the USEPA selected sediment removal as a method to reduce PCBs in the system (USEPA 2002). Sediment removal began in the upper 6 mile section of the site in May 2009. PCB concentrations in the upper Hudson River generally decrease with distance downriver, with the highest concentrations located closer to the points of initial discharge (USEPA 2000).

As long-lived mammals with relatively slow reproductive rates, bats are potentially sensitive to environmental contamination through repeated exposure and accumulation (Clark and Shore 2001; Rowe 2008; Secord et al. 2015). The nine species that occur along the upper Hudson River are insectivorous and some, such as *Myotis lucifugus* (little brown bats; LeConte,

1831), feed mainly over water and eat many aquatic insects (Clare et al. 2011). Other species, such as *Lasiurus cinereus* (hoary bats; Beauvois, 1796), feed on larger insects usually captured higher above ground level (Hickey and Fenton 1996). Bats can accumulate contaminants, such as PCBs (Clark and Shore 2001; Hickey et al. 2001) from contaminated sediments by consuming insects with sediment-based larval stages (Larsson 1984; Maul et al. 2006; Kannan et al. 2010). Insect species from the Orders Diptera, Ephemeroptera, and Trichoptera, are frequent prey of insectivorous bats, and in some cases are tolerant of contamination and can accumulate PCBs from contaminated aquatic environments (e.g., Reinhold et al. 1999; Larsson 1984; Kovats and Ciborowski 1989; Yu et al. 2013). Lilley et al. (2012) found that although chironomid species richness decreased with increasing sediment contamination, the overall biomass of chironomids did not change with increasing contamination (Lilley et al. 2012), providing opportunity for riparian predators to consume insects with high levels of contamination. Elevated levels of PCBs within the tissues of *M. lucifugus* have been reported from the Hudson River (Hudson River Natural Resource Trustees [HRNRT] 2007). Additionally, based on estimated daily dietary intake of PCBs for *M. lucifugus*, the USEPA (2000) reported that the survival and reproductive capability of *M. lucifugus* in the upper Hudson River may be impaired due to PCB exposure.

To investigate the effects of historical PCB contamination on bat activity and foraging behaviour in the wild, we monitored echolocation calls of wild bats and used them as an indicator of population composition in areas that varied in historical PCB contamination. Additionally, we radio-tracked *M. lucifugus* and measured insect abundance at sites where we collected bat activity data to determine the effects of historical PCB contamination on bat foraging behavior and prey availability. We tested three predictions. First, that bat activity decreases with increasing PCB sediment concentration; second, that insect abundance remains

the same regardless of PCB concentration, and; third, that foraging times and distances of *M. lucifugus* roosting along the Hudson River would not be significantly different from *M. lucifugus* roosting in an area without historical PCB contamination.

Methods

Our work was conducted under Permit 1279 from the New York Department of Environmental Conservation. From May through August of 2008 and 2009, we acoustically monitored bat activity at six sites and 48 sampling stations along a 40 km stretch of the Hudson River to determine bat population composition (Fig.1). The 48 sampling stations, or points where acoustic monitoring and insect sampling occurred, were grouped into the larger sites based on which stations were sampled within the same night. One site, consisting of several sampling stations, was monitored each night (see Table 1 for list of sampling sites and stations). To determine bat foraging duration and the distance each bat travelled nightly, we radio-tracked 35 *M. lucifugus* from two roosts along the Hudson River in 2008 and 2009, as well as a control roost not on the river (approximately 120km north of the Hudson roosts) in 2009.

The stretch of the Hudson River we monitored contains a wide range of PCB-contaminated sediment, even within small areas (Table 1). The National Oceanic and Atmospheric Administration (NOAA) database contains data on sediment sampling studies conducted on the Hudson River. We used the NOAA database to obtain PCB concentration levels from surface sediments of the Hudson River (NOAA 2013), from sites as close as possible to our sampling stations. To remain consistent, all of the values we obtained were from sediment samples taken by GE in 2003.

Acoustic sampling

Each night we began recording once we confirmed the presence of the first bat (either acoustically or visually) and continued to record for 10 minutes before moving to the next sampling station. We did not record on nights when temperatures were $< 10^{\circ}\text{C}$, or when strong winds ($> 4\text{ m}\cdot\text{s}^{-1}$) and heavy rain persisted. We recorded echolocation calls using an Avisoft-Bioacoustic CMPA/CM16 unit equipped with four condenser microphones (UltraSoundGate 416-200; Avisoft Bioacoustics, Berlin, Germany). Calls were recorded simultaneously onto four separate channels at a sampling rate of 250 kHz with 8-bit sample resolution, and automatically separated into one-minute files. These microphones which detected bat echolocation calls over about 40 m (Adams et al. 2012) were arranged in a tetrahedron shape to allow for a greater probability of call detection. There was 1 m between each microphone. Three of the microphones were pointed parallel to the river, while the fourth was pointed directly upwards to capture the calls of bats flying directly above our location.

To assess levels of bat activity we analyzed recordings made with the microphone array using Avisoft-SASLab Pro (version 4.3). To assess bat activity, we counted the number of search-phase bat passes (equal inter-pulse intervals between calls) present in each one-minute file from the microphone channel that recorded the most calls for each minute. Search-phase bat passes consist of a series of individual calls and are a commonly used metric to assess bat activity (e.g., Vaughn et al. 1997; Dzal et al. 2011; Jung et al. 2012). We identified bat species present at our study sites by specific parameters in their echolocation calls. Specifically, we looked at call duration, inter-pulse intervals, minimum and maximum frequency, frequency with most energy, and the presence of harmonics. These call parameters were then compared to values

in the literature for bat species present in New York State (Fenton and Bell 1981; van Zyll de Jong 1985; Obrist 1995).

Insect sampling

To test whether or not insect abundance was affected by PCB concentrations in our sampling area, we sampled insects at sites where we did acoustic sampling. Following methods similar to von Frenckell and Barclay (1987), we used traps made of 38 cm long, 11 cm diameter white PVC tubing, coated with Rotella[®] heavy-duty axle grease. Insect traps were suspended between 0.5-1 m above the surface of the water from trees along the shore of the Hudson River. Trap locations coincided with sampling stations that were acoustically sampled that night. Therefore, if Site 4 was being sampled, we set out 9 traps (one for each sampling station). We identified insects collected from traps to insect Order. When we could not identify an insect due to missing anatomical parts, we labelled it as “unknown”. While the traps were generally out from dusk until dawn, the actual number of hours varied slightly due to extraneous factors (e.g., weather). We accounted for the number of hours each trap was out by dividing our insect counts by the total number of hours the traps were out for each night. Our analysis was based on the total number of insects comprising three orders (Diptera, Trichoptera, and Ephemeroptera) because they made up the majority of captures (> 95% in both years).

Radio-tracking

We tracked a total of 35 *M. lucifugus* from three different roosts: two roosts along the Hudson River (2008 and 2009) and a control roost (2009) 120 km north of Hudson Falls (Fig.1). In 2008, all radio-tracked bats roosted in buildings along the Hudson River: 10 bats in Fort Miller, NY (43°09'49.1"N, 73°34'55.7"W), and 6 in Greenwich, NY (43°07'14.6"N, 73°34'39.0"W). In 2009, we tracked individuals from Fort Miller and Greenwich (4 and 10 individuals,

respectively) and 5 individuals from a control roost away from the Hudson River in Willsboro, NY (44°21'38.97"N, 73°23'30.71"W). We tracked bats for a total of 49 days (303 bat nights) between the first week of June and mid-August in 2008, and 57 days (233 bat nights) in 2009. We defined a bat night as each night a bat carried an active transmitter. Most of the individuals that we radio-tracked (28/30) were reproductive adult females; however, to increase our sample sizes we also tracked five subadults and two non-reproductive females (Table S1).

We captured *M. lucifugus* for radio-tracking by setting mist-nets near or within maternity roosts, or by entering the roost and taking bats off the wall. We attached a LB-2N radio transmitter (Holohil Systems, Carp, ON, Canada) to the bat using surgical cement (Torbot Bonding Cement, Torbot Group Inc., Cranston, Rhode Island, USA) and placed the transmitter between the bats' scapulae. We ensured that the transmitter weighed 6% or less of the bat's body mass in order to minimize its effects on flight maneuverability (Aldridge and Brigham 1988).

Following methods similar to Fenton and Rautenbach (1986), we tracked bats by foot, car and boat using a telemetry receiver. We began tracking every night one hour before sunset and completed tracking between 1 am and sunrise. For each radio-tracking bout (i.e., period after each capture event when all bats with transmitters were monitored until the last transmitter's battery life ended, or the transmitter fell off) we ensured that there were at least two instances in which we tracked through the entire night. To receive transmitter signals, we used three Lotek SRX-400 scanning receivers (Lotek Engineering Inc., Newmarket, ON, Canada), attached to either an AN-ADH 174 antenna (Lotek Engineering Inc., Newmarket, ON, Canada) or a F172-3FB antenna (AF Antronics Inc., Urbana, IL, USA). General locations of individuals were identified by the loudest signal. One team of trackers remained stationary and monitored signal strength while the second team travelled by car or boat to follow the signals. Signal strength and

times of recording were compared between the two teams and the Lotek SRX-400 receiver that remained in the roost at all times. This device automatically scanned for tagged bats every 10 minutes and stored transmitter pulse rate (and thus bat presence) for each individual when it was within range. Maximum foraging distance was defined as the maximum distance from the day roost we recorded signals from a tagged bat.

To determine foraging duration, we measured the time tagged bats were out of the roost as indicated by the receiver inside the roost. Because some bats use night roosts in addition to their day roosts, we followed bats throughout the night and subtracted time spent night roosting from the total foraging time. We considered a bat to be night roosting if it spent more than one minute continuously in one specific area.

Statistical analysis

Bat activity and insect abundance

We used R statistical software (version 3.1.2) for all statistical analyses (R Core Team 2014). We used a generalized linear mixed model ($GLMM_{admb}$) from the “glmmADMB” package (Skaug et al. 2006) with a negative binomial distribution to test for differences in bat activity levels (number of bat passes per 10 minutes) as well as insect abundance (number of insects per hour) with varying PCB concentrations along the Hudson River. We used a mixed model with site as a random effect to account for the individual stations within each site (i.e., stations sampled on the same day), and for repeated sampling at the same stations between May-August and among years. We included Julian day, temperature, and wind speed as covariates in both analyses, to control for any effect they may have had on bat activity. Additionally, we included minutes after sunset as a covariate in the analysis of bat activity to control for the inherent differences in bat activity as the night progressed. We used the surface sediment PCB

concentration at each of the 48 sampling stations as the main predictor variable in the analysis (NOAA 2013).

Bat foraging behaviour

We used a Gaussian mixed effects model with a random structure (*lme*, in the “nlme” package; Pinheiro et al. 2013) to determine if there were differences in the maximum distances travelled by foraging *M. lucifugus* among roosts (two roosts along the river and one control roost away from the river) and between years. After comparing model Akaike Information Criterion (AIC) values (Akaike 1974), we concluded that it was not necessary to include Julian day as a random intercept and used a linear regression model (*lm*, in the “nlme” package) to determine if there were differences in time spent foraging by *M. lucifugus* among roosts and between years.

Results

Bat Activity

Two species, *M. lucifugus* and *L. cinereus*, accounted for 52.3% and 41.0%, respectively, of bat activity recorded along the Hudson River (Table 2); consequently, we restricted detailed analyses to them. There were no differences in activity levels of either *M. lucifugus* ($z = 0.38$, $p = 0.706$) or *L. cinereus* ($z = -0.05$, $p = 0.960$) in relation to sediment PCB concentration.

Insect availability

Insect abundance along the Hudson River did not change with sediment PCB concentration ($z = 0.16$, $p = 0.873$).

Foraging behaviour

Overall, roost location was the only variable that significantly affected the time individual *M. lucifugus* spent foraging ($F = 36.45, p < 0.001$) as well as the maximum distances foraging *M. lucifugus* ($F = 4.20, p < 0.05$) travelled (Figure S1). The average foraging durations of radio-tagged *M. lucifugus* ranged from 103 ± 34 minutes a night to 502 ± 22 minutes a night (Table S1). Average distance travelled by *M. lucifugus* from roost location to foraging site ranged from 0.32 ± 0.01 km to 2.81 ± 0.13 km, but data for individual bats showed an overall distance range of 0.14 km and 4.11 km².

Bats from the control roost (no historical PCB contamination) foraged longer than bats from either roost along the Hudson River (Fort Miller roost: $t = 6.73, p < 0.001$; Greenwich roost ($t = 6.64, p < 0.001$) (Fig. 2). Additionally, bats from the control roost travelled farther to foraging sites than bats from the Fort Miller roost ($z = 2.67, p < 0.05$; Fig. 3) but not Greenwich ($z = 1.57, p = 0.25$; Fig. 2). There were no differences in foraging duration ($t = -0.898, p = 0.64$) or foraging distances ($z = 1.74, p = 0.18$) between bats from roosts with varying levels of historical PCB contamination located on the Hudson River.

Discussion

While bats roost and forage in areas of the upper Hudson River that have been historically exposed to PCBs, these contaminants appear to have no adverse effect on bat activity or behaviour. We found no statistically significant relationship between historical PCB contamination and the activity of bat species examined, nor an effect on insect abundance at sites along the upper Hudson River. These findings did not support our first prediction that the activity levels of *L. cinereus* and *M. lucifugus* would decrease with increasing PCB concentrations, but did support our second prediction that insect abundance would be the same regardless of

sediment PCB concentration. We found significantly longer foraging times and distances for *M. lucifugus* roosting in areas with no historical PCB contamination (control) compared to those roosting in areas with high historical PCB contamination, which did not support our third prediction.

Fukui et al. (2006) found that flux of aquatic insects emerging from streams is one of the most important predictors of bat activity. Therefore, it is possible that the Hudson River supports more insect prey than the foraging area at the control site in Willsboro. This could explain differences in foraging times and distances between the two areas.

M. lucifugus and *L. cinereus* activity along the Hudson River were comparable to levels recorded at other uncontaminated sites in North America (Brooks 2011; Ford et al. 2011). Furthermore, while bats along the upper Hudson River are likely to be exposed to PCBs trapped in sediment through the insects that they consume, several factors could mitigate the impact of exposure. First is the high level of variability in sediment PCB concentrations at sites along the upper Hudson River. Although sediment PCB concentrations are generally highest closest to the sources of contamination (USEPA 2000; Fig. 1) and gradually decrease downriver, concentrations vary widely even within a small area (NOAA 2013). Second, foraging bats can travel long distances each night. *M. lucifugus* can travel > 2.5 km from roost to foraging sites (Henry et al. 2002), while the larger *L. cinereus* can travel up to 20 km (Barclay 1989). Both species roosting along the Hudson River could, therefore, easily forage over contaminated and uncontaminated stretches of river on any night.

Flexible foraging behaviour may make many bat species resilient and resistant to local changes in the distribution of roosts and prey, such as those associated with the impact of contaminants. Many species of insectivorous bats are opportunistic foragers, quick to exploit

local concentrations of insects such as those emerging from water (Clare et al. 2011; Clare et al. 2014) or attracted to lights (Fenton and Morris 1976; Bell 1980). Opportunistic insectivorous bats tend to eat a wide range of insects, typically focusing on the largest insects they can handle and that they most often encounter when foraging (e.g., Barclay 1985). *Lasiurus cinereus* and *M. lucifugus*, the species most active at our sampling sites along the Hudson River, are opportunistic in their prey selection and have broad diets (Belwood and Fenton 1976; Anthony and Kunz 1977; Barclay 1985; Clare et al. 2011). Therefore, they eat a variety of insect species, belonging to many orders, even those associated with water of poor quality (Clare et al. 2014). Indeed, some species of insectivorous bats can tolerate degraded habitats, especially if the degraded habitats increase the availability of certain insects (Kalcounis-Rueppel et al. 2007). Kalcounis-Rueppel et al. (2007) found that activity of the insectivorous bat *Perimyotis subflavus* (tri-colored bat; Cuvier, 1832) was higher downstream compared to upstream of a wastewater treatment plant, likely due to the increased levels of trichopterans and hymenopterans, which make up a large proportion of the diet of *P. subflavus*.

Some vertebrates develop resistance to contaminants, such as heavy metals (Shore and Douben 1994; Wirgin et al. 2011). Common shrews (*Sorex araneus*; Linnaeus, 1758) can store high levels of cadmium within their organs with no adverse effects (Shore and Douben 1994). Bottlenose dolphins (*Tursiops truncatus*; Montagu, 1821) contain an immobile fatty tissue (melon) that can accumulate large concentrations of persistent organic contaminants, effectively isolating the contaminants from tissues that are susceptible to toxic effects (Yordy et al. 2010). In fact, PCB resistance has also been observed in species inhabiting the Hudson River. Atlantic tomcod (*Microgadus tomcod*; Walbaum, 1792) have evolved a genetic resistance to PCB contamination (Wirgin et al. 2011). Similarly, Cohen et al. (1991) found that PCB-resistant

strains of two species of marine diatoms accumulate less PCBs than sensitive strains (Cohen et al. 1991). To our knowledge, resistance to PCBs has not been documented in any mammal studied to date. However, bats have been documented tolerating high levels of other chemicals (Lilley et al 2013). Daubenton's bats (*Myotis daubentonii*; Kuhl, 1817) have been documented with high levels of organic tin compounds within their fur and with no apparent physical or physiological stress beyond immunosuppression (Lilley et al. 2013).

We conclude that historical contamination of the upper Hudson River with PCBs has not had a negative effect on the activity and foraging behavior of bats, nor on the availability of their insect prey. The high activity levels of bats that we recorded along the upper Hudson River indicate that bat species and their local insect prey have flourished for many years despite historical PCB contamination of the river. Although bat activity and foraging behaviour along the Hudson River do not appear to have been effected by PCB contamination, the accumulation of contaminants by bats worldwide is an increasing concern. Aquatic environments are being flooded with a variety of contaminants of emerging concern (CECs), such as pharmaceuticals and personal care products (Secord et al. 2015). High concentrations of a variety of contaminants (e.g., polybrominated diphenyl ethers, salicylic acid, and caffeine) are found in bats throughout the northeastern United States, which may have detrimental consequences when combined with other factors such as White Nose Syndrome (Secord et al. 2015). Future work should further examine the effect of CECs on local bat populations, and the potential interaction that CEC and PCB concentrations could have on bats foraging along rivers.

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Table 1. Concentrations of total PCBs (parts per billion [ppb]) within the surface sediment of the Hudson River, at the sites and sampling stations where we conducted acoustic monitoring of bats.

Site-Station	Total PCB concentration (ppb)	Site-Station	Total PCB concentration (ppb)
1-1	6440	4-1	2300
1-2	1920	4-2	3400
1-3	2040	4-3	1860
1-4	10360	4-4	380
1-5	3400	4-5	1000
1-6	5840	4-6	1650
2-1	3300	4-7	12500
2-2	1640	4-8	650
2-3	4300	4-9	27000
2-4	980	5-1	3000
2-5	2160	5-2	2360
2-6	2550	5-3	4080
2-7	1040	5-4	370
2-8	1900	5-5	4600
2-9	1710	5-6	23000
2-10	6100	6-1	19400
3-1	10500	6-2	13400
3-2	970	6-3	2630
3-3	2970	6-4	231000
3-4	750	6-5	33000
3-5	4500	6-6	10600
3-6	5490	6-7	124000
3-7	2500	6-8	27000
3-8	4900	6-9	82000

Table 2. The total number of passes recorded for each bat species at the six sampling sites along the Hudson River in 2008 and 2009.

Bat species	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	All Sites
<i>Myotis lucifugus</i> , little brown bat	870	1161	352	622	922	1213	5140
<i>Lasiurus cinereus</i> , hoary bat	772	663	465	928	536	660	4024
<i>Eptesicus fuscus</i> , big brown bat	9	31	4	25	80	21	170
<i>Perimyotis subflavus</i> , tri-coloured bat	70	97	18	82	45	14	326
<i>Lasiurus borealis</i> , Eastern red bat	72	53	1	17	19	6	168
All species	1793	2005	837	1674	1602	1914	9825

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Fig. 1. Sampling area along the Hudson River, NY. Roost sites used for radio-tracking are indicated by the filled stars; sites of the original contamination sources are indicated by triangles.

Fig. 2. Time spent foraging at each maternity roost. Mean time *Myotis lucifugus* (little brown bat) spent foraging ($\pm$ standard deviation) was higher in the control location than in either location along the Hudson River. Significant differences are denoted with letters. Data from the control location ($n = 5$) were from 2009 only, whereas data from the Hudson River locations (Fort Miller, $n = 13$; Greenwich, $n = 16$) includes 2008 and 2009.

Fig. 3. Foraging distances at each maternity roost. *Myotis lucifugus* (little brown bats) travelled farther (mean foraging distance $\pm$ standard deviation) to forage at the control site than at the Fort Miller roost, although there were no significant differences between either the Fort Miller roost or the Greenwich roost, or the Greenwich roost and the control site. Significant differences are denoted with letters. Data from the control location ($n = 5$) was from 2009 only, whereas data from the Hudson River locations (Fort Miller, $n = 13$; Greenwich, $n = 16$) includes 2008 and 2009.

Sampling area along the Hudson River, NY. Roost sites used for radio-tracking are indicated by the filled stars; sites of the original contamination sources are indicated by triangles.
279x215mm (300 x 300 DPI)

Time spent foraging at each maternity roost. Mean time *Myotis lucifugus* (little brown bat) spent foraging (+/- standard deviation) was higher in the control location than in either location along the Hudson River. Significant differences are denoted with letters. Data from the control location (n = 5) were from 2009 only, whereas data from the Hudson River locations (Fort Miller, n = 13; Greenwich, n = 16) includes 2008 and 2009.

248x182mm (300 x 300 DPI)

Foraging distances at each maternity roost. *Myotis lucifugus* (little brown bats) travelled farther (mean foraging distance +/- standard deviation) to forage at the control site than at the Fort Miller roost, although there were no significant differences between either the Fort Miller roost or the Greenwich roost, or the Greenwich roost and the control site. Significant differences are denoted with letters. Data from the control location (n = 5) was from 2009 only, whereas data from the Hudson River locations (For Miller, n = 13; Greenwich, n = 16) includes 2008 and 2009.

249x184mm (300 x 300 DPI)