Supplement for:
Pintar, Matthew R., and Brian J. Olsen. Landscape acidification has trophic-mediated effects on Ovenbirds (*Seiurus aurocapilla*). The Wilson Journal of Ornithology.

Territory mapping
From 14 May–17 July 2010 we conducted spot mapping (International Bird Census Committee 1970) of Ovenbird territories by walking throughout BBWM and adjacent areas from ~05:00–10:00 am. Male movement, calling, and interactions with conspecifics were tracked, and song posts were marked with a handheld GPS unit (Garmin eTrex H, Lenexa, KS). All known territories were found by late May, and after early June, mapping efforts focused on the areas with known territories, with occasional visits to areas without known territories. Mapping was largely completed by the first week of July (389 total song posts), with only a limited number of points collected in mid-July (12 song posts).

Song posts and flight paths that overlapped from locations marked on different days were assumed to be the same bird. Observations of different males made at the same time were disconnected from each other, and borders were placed near locations of counter-singing males. Most observations with no connection to other observations were assumed to be different birds unless the proximity was very close ($< \sim$20 m) to a known territory or if no other territory was in the area. Territories were distinguished from each other by plotting all points on one map in ArcGIS 9.3 and connecting simultaneous observations of the same male. Territories that were completely within East Bear or completely off of both watersheds were considered non-acidified, control territories (N = 4), while those on West Bear were acidified territories (N = 3). One territory was situated on the border of both watersheds and was considered a mixed territory and excluded from the categorical (acidified vs. control) territory analyses. Territories are mapped on Fig. S1.

Territory size was determined in ArcGIS using 2 minimum convex polygon (MCP) estimates: one that excludes song posts disconnected from the core of a territory (MCP1), and a second, less conservative method, that includes those points (MCP2). This resulted in differences in MCP areas for four of the eight territories. We tested for differences in mean territory area between watersheds using mixed effects models with watershed as a fixed effect and mapping effort (number of days males were recorded at each territory) as a random effect fit by maximum likelihood with the Satterthwaite method using R v 4.1.1 with the *lme4* v 1.1-27.1 and *lmerTest* v 3.1-3 packages (Bates et al. 2015, Kuznetsova et al. 2017, R Core Team 2021).

In addition to minimum convex polygons, we initially used the kernel density estimator from Hawth's Analysis Tools for ArcGIS (Beyer 2004) for fixed kernel density analysis with the smoothing factor calculated using an ad hoc method described by Worton (1989). The mean smoothing factor calculated across all eight territories was used in the fixed kernel density estimates because four of the 8 territories did not contain the minimum 50 points per territory recommended by Seaman et al. (1999). A raster cell size of 1 m^2 was used along with a scaling factor of 1,000,000 and percent volume contours of 50, 90, and 95, which produced three areas in each territory for 50, 90, and 95% confidence, respectively. All points used in the MCP2 method were used in the kernel density analyses. We corrected for the number of observations because of the inadequacy of our sampling for traditional kernel density estimation methods. Only half of our 8 Ovenbird territory kernel density estimates used sample sizes that exceeded the recommended 50 points, and 2 of the 4 undersampled territories used less than the minimum

of 30 points recommended by Seaman et al. (1999). We were worried that our territory estimates might be biased large because of the inability of our kernel estimator to distinguish outlying points given our low sampling size. While preliminary results indicated kernel density analyses were similar to minimum convex polygon analyses, we chose to exclude them due to the aforementioned limitations.

Leaf litter arthropods
To assess the abundance, community composition, and calcium concentration of available prey, we collected leaf litter samples from within known territories. Two samples were taken from each of seven territories in the morning on three dates: 8, 13, and 17 July, providing a total of 6 invertebrate samples from each territory during a period of possible offspring provisioning. While these samples were collected later than the nests we observed (mid-June; see below), it is still possible some birds may be provisioning for their offspring, especially fledglings, at this time. Regardless, we may expect arthropods at this time to be somewhat representative of differences during the prior 2 months, particularly with regard to calcium concentrations.

The territory outside of either watershed was excluded from arthropod sampling as birds were not observed there during July, while the territory spanning the border of both watersheds had one sample collected from each watershed on each sampling date. We attempted to distribute the six arthropod sampling sites evenly across the core of each territory (where we most often observed males calling), avoiding non-leaf litter objects (rocks, logs, etc.). Leaves from a 900-cm^2 area were collected to a depth at which it seemed adults would reasonably be able to forage (~5 cm depending on litter compaction); typically loose leaf litter, similar to Zach and Falls (1979), but without collecting from above the leaf litter. Densely compacted leaf litter, in addition to material that was part of the soil's O horizon, was not collected. Samples were immediately transported to the lab and placed in Berlese funnels for 48 h, with arthropods preserved in 75% ethanol.

Arthropods greater than 5 mm long were deemed large enough to be food (Stenger 1958, Zach and Falls 1978, Holmes and Robinson 1988), with insects identified to order (Coleoptera, Diptera, Hemiptera, Lepidoptera) and Myriapoda identified to the groups Chordeumatida, Geophilomorpha, Julida, and Polydesmida. Although we did not expect Ovenbirds to regularly feed on Myriapoda, we included them here for illustrative and comparative purposes because they were relatively abundant in our samples and may be calcium sources within the food web. Highly abundant but tiny arachnids (most <1 mm, spiders and mites) were assessed separately for community and abundance metrics only, not because they would be typical food items.

We tested for differences in abundances of each of 8 commonly sampled (total abundance ≥ 30) arthropod groups (all insects, Coleoptera larvae, Coleoptera adults, Diptera larvae, Myriapoda, Geophilomorpha, spiders, mites) between watersheds using separate univariate mixed effects analyses with watershed and elevation as fixed effect and collection date as a random effect. We included both sampling date and elevation within each watershed as covariates because we expected both the effect of acidification and invertebrate community characteristics to vary with these factors independent of acidification (Lessard et al. 2011). We also used permutational analysis of variance (PERMANOVA) to assess whether there were differences between watersheds in the structure of the overall arthropod assemblages (insects and myriapods, excluding arachnids) and insect assemblages (insects only, excluding myriapods and arachnids). Our PERMANOVA also included watershed and elevation as fixed effects, and it used Bray-Curtis distances as the distance between community space, 9999 permutations to

estimate the null distance, and set collection date as our sampling strata using the *adonis* function with the *vegan* package v 2.5-7 (Oksanen et al. 2021). Arthropod abundance and community analyses were conducted on individual leaf litter samples (N = 42).

We collected a total of 154 insects and 80 myriapods; we found no significant differences in abundances of any individual groups of arthropods between watersheds (Table 2). All insects, Coleoptera adults, and Coleoptera larvae were more abundant at higher elevations, while spiders were more abundant at lower elevations (Table 2). We found no differences in overall assemblage structure between watersheds using Bray-Curtis dissimilarity for either total arthropods ($F_{1,39} = 0.98$, $P = 0.38$) or insects ($F_{1,39} = 1.02$, $P = 0.31$), but both total arthropods ($F_{1,39} = 3.32$, $P = 0.01$) and insects ($F_{1,39} = 3.87$, $P = 0.01$) significantly varied with elevation.

Because of low overall total mass, only four groups were abundant enough to test for calcium: Coleoptera adults, Coleoptera larvae, Diptera larvae, and Geophilomorpha. These samples were dried at 60°C for 48 h in glass scintillation vials to remove excess water. Due to detection limits, only 1 aggregate sample of each of the groups Geophilomorpha, Diptera, and Coleoptera larvae were tested from each watershed (all individual samples from each watershed were combined). For the most commonly sampled group (Coleoptera adults) we were able to test one aggregate sample from each territory (all individual samples combined within each territory). Arthropod samples were tested for calcium concentration by atomic absorption spectroscopy at the University of Maine Analytical Laboratory and Soil Testing Service. Samples were prepared using the dry ash method and diluted to 25 ml total volume. Detection limits for Calcium were 0.1 mg Ca per 1 mL of solution.

Dry ash weights of adult Coleoptera samples ranged from 0.002–0.056 kg, and the 2 lowest values (0.002 kg, 0.006 kg) were below detection limits and had their Calcium concentrations of adult Coleoptera samples returned results as a range representing the maximum possible observed value, rather than an exact concentration. For both of these samples we assigned the value of the detection limit. One of these concentrations was excluded from analyses as its maximum value (0–1250 mg/kg) was much higher than all other concentrations and thus encompassed a wide range of uncertainty; this sample also had the lowest dry weight (0.002 kg). The maximum value for the second concentration (0–416 mg/kg) was lower than all other concentrations, and so we maintained the maximum value (416 mg/kg) in our analyses as a conservative estimate; this sample had the second lowest weight (0.006 kg). To assess differences in calcium concentrations of adult Coleoptera between watersheds, we used a t-test. We also assessed whether birds may be responding to calcium availability by using a mixed effects models to compare territory areas (using all five area estimates) to the calcium concentration of the Coleoptera adults from the territory in which they were collected, with number of days males were observed as a random effect fit by maximum likelihood with the Satterthwaite method. We initially included adult Coleoptera abundance as a covariate in this analysis, but it was not significant and was dropped from the model.

Across the aggregate samples of Coleoptera larvae, Diptera larvae, and Geophilomorpha we collected from each watershed, calcium concentrations trended lower in the acidified watershed for all three groups. Calcium concentrations were lower in Coleoptera adults than any other taxonomic groups tested, and mean calcium concentrations in Coleoptera adults were not different between watersheds ($F_{1,3} = 0.04$, $P = 0.86$). When controlling for number of male observations, Ovenbird territory area was significantly inversely related to calcium concentration in adult Coleoptera for both MCP methods (MCP1: $F_{1,6} = 97.6$, $P < 0.0001$; MCP2: $F_{1,6} = 7.5$, $P = 0.03$), showed marginal relationships using KD 90 ($F_{1,6} = 4.8$, $P = 0.07$) and KD 95 estimates

($F_{1,6} = 5.3$, $P = 0.06$), and no significant relationship with the KD 50 estimate ($F_{1,6} = 2.6$, $P = 0.16$). We also conducted exploratory analysis with elevation as a covariate in analyses of territory size, but doing so did not change main effects of treatment or calcium.

Nestlings

We searched for nests by observing behavioral cues of breeding, but only 1 Ovenbird nest was found in each watershed. The West Bear (acidified) nest was monitored daily from egg-laying to chick fledging, and the nest on East Bear was monitored from its discovery with four-day-old chicks (age estimated using Jongsomjit et al. [2007]) until fledging. Chick mass, length of culmen, width of bill at nares, depth of bill at nares, length from nares to tip of bill, length of tarsi, minimum width of tarsi, and wing chord were measured daily until fledging. Mass was initially measured with an electronic scale (1 g accuracy), but was changed to a spring scale (0.25 g accuracy) on day 5 of measurements from the acidified watershed (all control watershed data used the spring scale). Length measurements were taken using Vernier calipers (0.005 mm accuracy). Only a single nest was found on each watershed and the chicks were not marked.

The single nest we found on the acidified watershed contained five eggs, all of which hatched between our visits on 7 and 8 June 2010 (day 1), but two of the nestlings disappeared between days four and five. On the control watershed, the nest was found when the nestlings were already four days old (estimated age on 13 June 2010). There was one unhatched egg in the nest along with four chicks, and all four survived until fledging at an estimated age of eight days. In both nests, the nestlings left the nest by the eighth day following hatching, which is typical of Ovenbirds (Stodola et al. 2010). Average nestling mass per nest (watershed) is shown in Fig. S2. Given our small sample size and inability to control for individual nestling effects, we do not consider nestlings further.

Data availability

Data associated with this study have been deposited in Figshare (Pintar and Olsen 2021).

Table S1. Mean (± SE) abundance per sample and results of mixed effects analyses for common arthropod groups in leaf litter samples between 2 watersheds and by elevation in Ovenbird territories at Bear Brook Watershed, Maine, in 2010. Total number of samples = 42. Bold indicates statistically significant results ($P < 0.05$); italics indicates marginal results ($0.05 < P < 0.10$). Direction indicates whether that taxon had greater abundances at higher or lower elevations, or no statistical difference (NS).

	Abundance		Watershed		Elevation		
	Control	Acidified	F	P	F	P	Direction
All insects	3.1 ± 0.6	4.2 ± 0.6	2.18	0.15	7.95	**0.01**	Higher
Coleoptera adults	1 ± 0.4	1.2 ± 0.3	0.05	0.82	6.51	**0.01**	Higher
Coleoptera larvae	1.5 ± 0.2	2.0 ± 0.3	0.77	0.38	6.82	**0.01**	Higher
Diptera larvae	0.5 ± 0.2	0.9 ± 0.2	3.33	*0.08*	0.02	0.89	NS
All myriapods	1.7 ± 0.4	2.1 ± 0.4	0.43	0.51	1.41	0.24	NS
Geophilomorpha	1.2 ± 0.3	1.8 ± 0.4	1.25	0.27	2.68	0.11	NS
Spiders	5.3 ± 0.9	6.8 ± 1.3	1.76	0.19	4.90	**0.03**	Lower
Mites	407.1 ± 36.0	330.2 ± 32.9	3.63	*0.06*	1.38	0.25	NS

Fig. S1. Map of territories (minimum convex polygon [MCP1] method; purple polygons), individual song posts (all points), and approximate watershed boundaries at BBWM. Elevation generally increases from southeast to northwest.
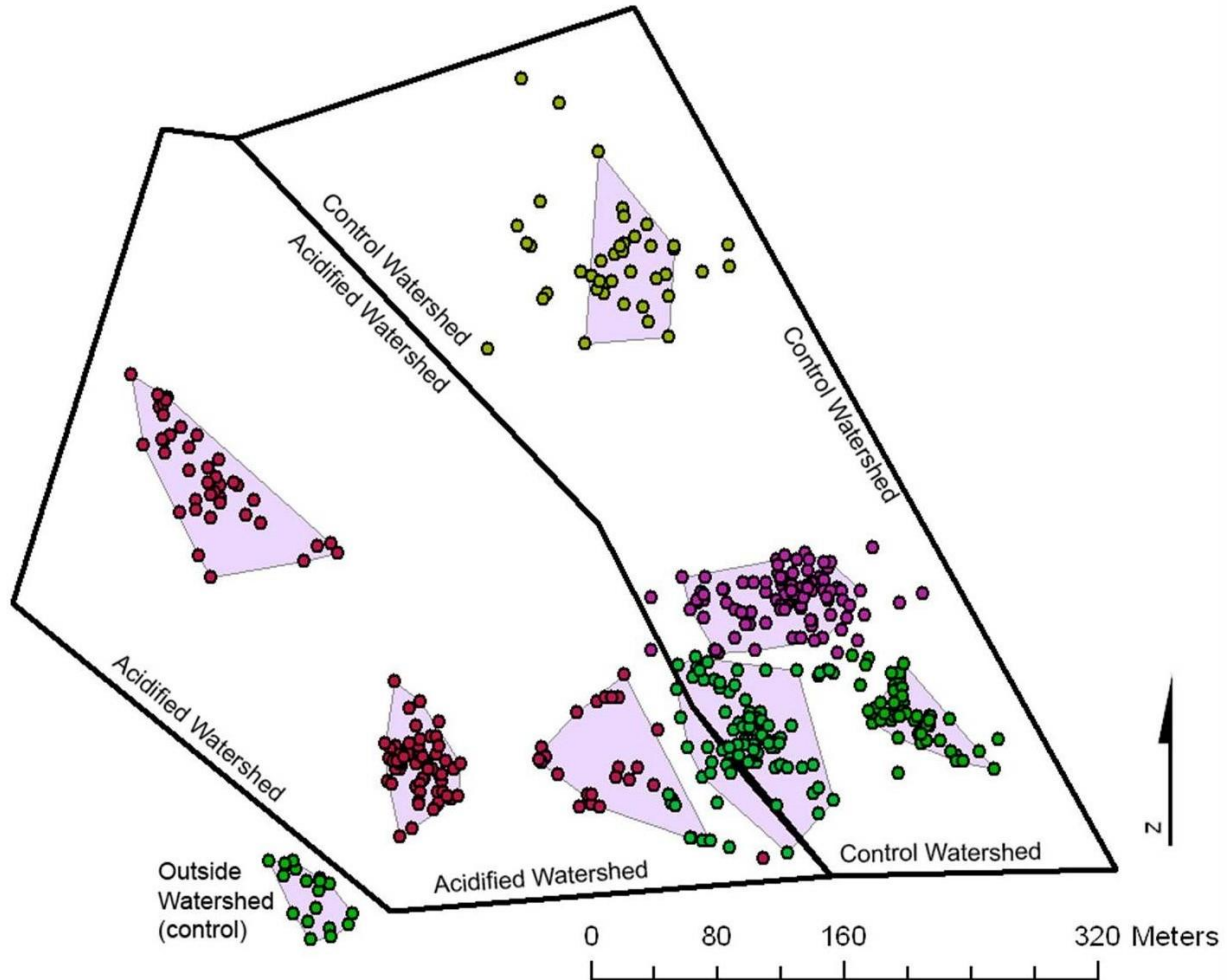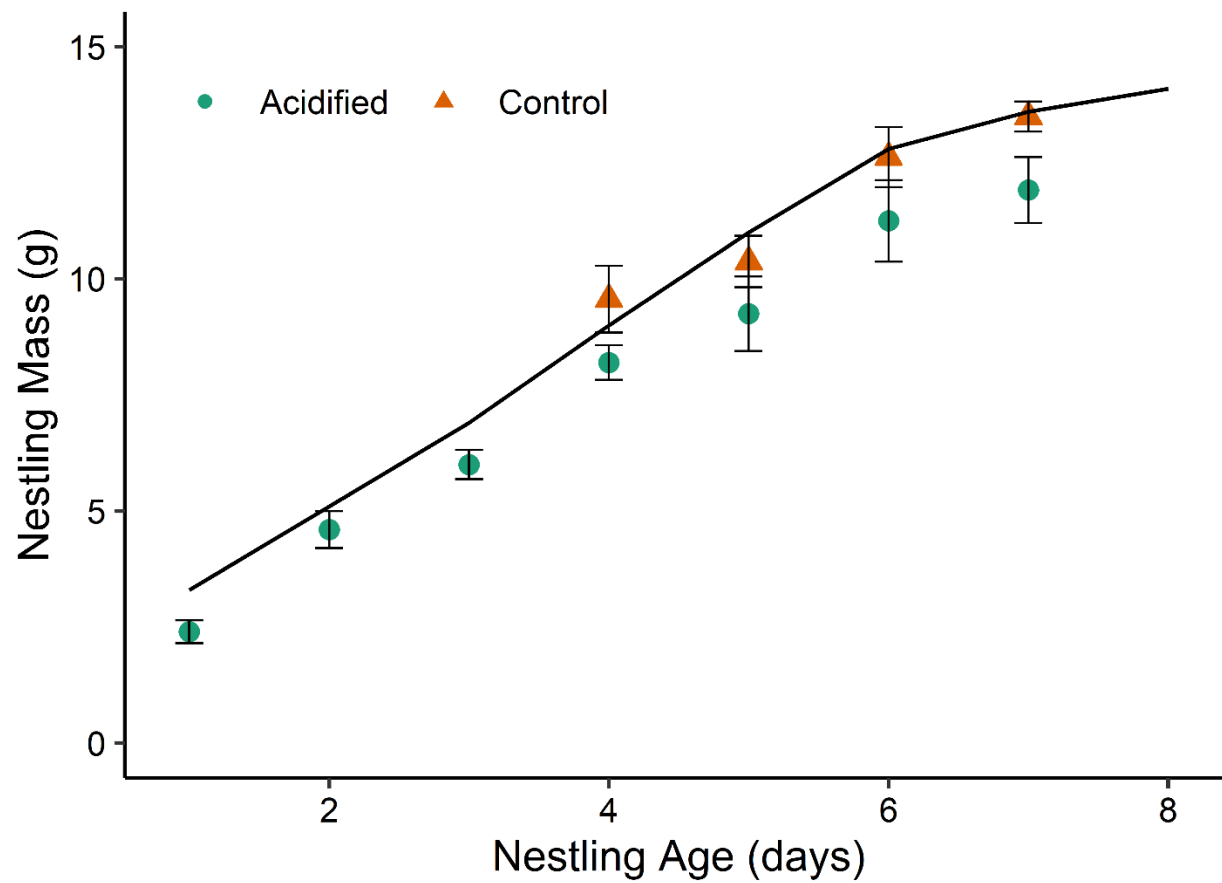
Fig. S2. Mean (± SE) nestling mass in the acidified and control watersheds by day until fledging. The black line represents Ovenbird nestling mass in a typical non-acidified landscape from Van Horn and Donovan (1994).

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