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The role of weather in mediating the effect of mercury exposure on reproductive success in tree swallows

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Abstract Mercury is a heavy metal that has contaminated countless ecosystems throughout the world. A large body of literature has documented reproductive, physiological, and behavioral impairments associated with mercury exposure in laboratory settings, but whether and how such effects are manifest in free-living populations remains poorly understood. The purpose of this study was to evaluate whether tree swallow (*Tachycineta bicolor*) breeding success at a site with high mercury exposure varied with ambient temperature or precipitation at various points in the breeding cycle. Tree swallows nesting along the South River had significantly elevated blood total mercury (mean ± SE: 3.03 ± 0.15 μg/g) compared to swallows breeding on reference sites (mean ± SE: 0.16 ± 0.005 μg/g). These high levels of mercury were associated with reduced hatching and fledging success, and contaminated birds produced approximately one less fledgling per nest than their reference counterparts. The magnitude of this difference was weather-dependent: unusually high ambient temperatures encountered early in the nestling period were associated with reduced reproductive output in contaminated, but not in reference, birds. In contrast, little effect of mercury on success of nestlings was observed when temperatures were cooler, and precipitation also had no detectable interaction with mercury. These results provide insight into mechanisms underlying reproductive effects of mercury. In addition, these findings underscore the importance of considering variable environmental conditions when assessing effects of contaminants on free-living wildlife. In particular, projections about the effects of global climate change on ecotoxicological impacts must take into account the kinds of weather-mediated effect demonstrated here.

Keywords Climate · Heavy metal · Mercury · *Tachycineta bicolor* · Tree swallow · Weather

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Introduction

Mercury is a heavy metal that has contaminated many ecosystems worldwide (United Nations Environment Programme 2002). Although mercury is a naturally occurring element, human activities have increased its availability to wildlife. Upon initial release into the environment, most mercury is in an inorganic form. However, biological and chemical processes readily transform inorganic mercury into organic methylmercury, which can bioaccumulate and biomagnify within food webs. Because methylation processes frequently occur in aquatic environments (Wiener et al. 2003), terrestrial food webs were long thought impervious to mercury exposure. Only recently has this paradigm begun to shift, with a growing number of studies now reporting bioaccumulation in non-piscivorous wildlife with no direct link to aquatic mercury point sources (Cristol et al. 2008; Rimmer et al. 2010).

Dosing studies on captive aquatic birds have repeatedly demonstrated the potential for mercury to impair avian reproduction (Heinz 1979; Hoffman and Moore 1979; Frederick and Jayasena 2010), but the extent to which adverse effects occur in free-living populations remains poorly understood. Evidence is now mounting for individual fitness effects, with a number of field studies reporting abnormalities in immune competence (Hawley et al. 2009), endocrine functioning (Franceschini et al. 2009;

Wada et al. 2009), and behavior (Hallinger et al. 2010) in mercury-contaminated songbirds. Such effects have the potential to alter reproductive success (Brasso and Cristol 2008); however, demonstrating that mercury is the specific cause of these individual fitness effects, and that these individual fitness effects result in reproductive impairment, has proven difficult.

Identifying mechanisms through which mercury might impair nesting success is complicated by the large number of environmental variables that influence reproduction in birds. Habitat structure and food availability have been linked to reproductive performance in several species (Zanette et al. 2006; Cornell and Donovan 2010; Martin et al. 2010), and climatic variables, such as temperature and precipitation, likely contribute an additional layer of complexity (McCarty and Winkler 1999; Nooker et al. 2005; Dawson 2008). For example, Saino et al. (2004) found that low temperatures in the period prior to laying were correlated with lower mass and quality of barn swallow (*Hirundo rustica*) eggs. Similarly, Ludwig et al. (2006) observed reduced survival among black grouse (*Tetrao tetrix*) nestlings when post-hatching temperatures were unseasonably cold.

To better understand the impact of mercury on free-living songbird populations, it is necessary to examine and interpret contaminant effects within the appropriate environmental context. Previous research has demonstrated the efficacy of such an approach. Gentes et al. (2006) found that reproductive effects among tree swallows (*Tachycineta bicolor*) nesting on reclaimed oil sands wetlands were more apparent in years with harsh weather. Conversely, Custer et al. (2003) found that the reproductive advantage enjoyed by reference tree swallows over those at PCB-contaminated sites disappeared in a season with several spells of cold and rainy weather; hatching success in that year was uniformly poor at all sites, irrespective of contamination (Custer et al. 2003).

Our objective was to build on an earlier study of tree swallows nesting along the mercury-contaminated South River (Virginia, USA). In that study, Brasso and Cristol (2008) documented reduced nesting success in first-time-breeding females in one (2006) of two breeding seasons studied (2005–2006; Brasso and Cristol 2008). We used a larger data set to test our hypotheses that (1) reproductive success would be negatively impacted on mercury-contaminated sites over two breeding seasons (2006–2007); and (2) any effect of mercury exposure on reproductive success would be modulated by weather conditions, specifically ambient temperature and precipitation. By examining simultaneously the effects of contamination level and weather on reproductive performance at each stage of nesting, we hoped to gain insight into potential mechanisms through which mercury impairs breeding.

Methods

Study species

The tree swallow is an insectivorous migratory songbird that breeds throughout much of North America (Robertson et al. 1992). Like many cavity nesting birds, tree swallows readily occupy artificial nest cavities, and can thus be recruited in large numbers to sites of a researcher's choosing. Adults consume a diet that is both terrestrial and aquatic in origin (Robertson et al. 1992; Mengelkoch et al. 2004; Brasso and Cristol 2008), and as the breeding season progresses, their foraging activities become increasingly restricted to the area immediately surrounding their nests (Mengelkoch et al. 2004). Thus, tree swallows provide an excellent window into contaminant availability at a local scale. For these reasons, and many others, the tree swallow is emerging as a model organism in ecotoxicology (McCarty 2002; Jones 2003).

Study sites

The South River is a tributary of the South Fork Shenandoah River that was contaminated with industrial mercury between 1929 and 1950 (Carter 1977). Despite the passage of 60 years, contaminant levels in fish and sediment have remained elevated (Carter 1977; Cocking et al. 1991; Virginia Department of Environmental Quality 2008). Recently, Cristol et al. (2008) documented high blood mercury levels in nearly all songbird species breeding within 50 m of river shoreline. The levels of mercury observed in many of these species rank among the highest ever reported in free-living songbird populations (Brasso and Cristol 2008).

Beginning in 2005, a nest box trail was established at 19 sites along the contaminated South River, as well as at 17 reference sites upstream of the point source or along two adjacent tributaries, the North and Middle Rivers, with no history of mercury contamination (centroid of study area: 38°10′ N, 78°59′ W; Fig. 1 in Cristol et al. 2008). Boxes were constructed following a popular bluebird nest box design (North American Bluebird Society 2010) and each was fitted with a "stovepipe" predator guard that reduced snake and mammalian predation. Boxes were placed approximately 25 m apart in cropland or pasture, within 50 m of river shoreline in 2005–2006, and up to 350 m into the floodplain in 2007. In 2005, 146 nest boxes were available. This number was increased to 296 and 361 before the breeding seasons of 2006 and 2007, respectively.

Nest monitoring

Boxes were monitored weekly beginning in early April of each year. At each visit, an observer recorded the amount and type of nesting material present, number of eggs laid, or number and age of nestlings. Tree swallows lay one egg per day until clutch completion (Robertson et al. 1992). Thus, it was possible to estimate a precise date of clutch initiation for nests observed during the laying cycle. If observation occurred following clutch completion, clutch initiation date was back-approximated using known hatching dates and an estimated incubation period of 14 days, beginning with the penultimate egg (Robertson et al. 1992). Nestlings were banded between 10 and 16 days of age, and were considered to have fledged if they were present in the nest until at least 18 days, and if the empty nest contained fecal remains typical of fledging tree swallows (Robertson et al. 1992).

Egg measurement

Eggs were measured in a subset of nests in both 2006 and 2007. For every nest, all eggs were measured; however, because eggs were not marked, we were unable to make inferences about laying order. Eggs were measured length- and width-wise to the nearest 0.1 mm using a pair of manual calipers. Egg volume (V) was then calculated according to the formula:

$$V = L \times W^2 \times 0.51$$

where L is the length, W is the width, and 0.51 is the volume coefficient constant (Hoyt 1979).

Capture and sampling

Adult tree swallows were captured inside their nest boxes during incubation or the nestling period either by hand or using one of two trapping methods (Stutchbury and Robertson 1986; Friedman et al. 2008). Sex was determined by the presence of a brood patch (in females) or cloacal protuberance (in males). Adult females exhibit delayed plumage maturation, enabling us to further classify them as either second-year (i.e., one year old, hereafter SY) or after-second-year (i.e., at least two years old, hereafter ASY). Upon capture, each adult was banded with a single United States Geological Survey aluminum band and a small blood sample was taken following methods described in Brasso and Cristol (2008).

Mercury analysis

Mercury analysis was performed at either the Trace Element Research Laboratory of Texas A&M University or at the College of William and Mary. Blood samples were analyzed for total mercury concentration on a Milestone® DMA-80 (see methods in Cristol et al. 2008). Approximately 95% of the mercury present in avian blood and feather is in the organic form (Evers et al. 2005; Wada et al. 2009); thus, total mercury concentration should provide a reasonable approximation of methylmercury. All mercury values are presented as wet/fresh weight (μg/g) concentrations. Quality assurances have been described elsewhere for the same set of mercury data (Hallinger et al. 2011).

Environmental variables

Weather data were acquired on-line from the National Climatic Data Center website (http://www.ncdc.noaa.gov). Specifically, we obtained daily maximum temperature, minimum temperature, and precipitation from the Staunton Water Treatment Plant weather station (38°11′ N, 79°05′ W; coop id: 448062), which was located in close proximity (~9 km) to the center of our study area (see "Study sites" section).

Statistical analyses

Mercury levels and effects on reproduction

To determine whether mercury exposure was associated with reduced reproductive success in breeding female swallows, we performed a series of General Linear Models (GLMs) using blood mercury level, clutch initiation date, clutch size, egg volume (pooled by clutch), proportion of eggs hatched, proportion of nestlings fledged, or number of fledglings produced as the response variable. In every analysis, year (2006 or 2007), age (SY or ASY), site type (contaminated or reference), and all two-way interaction terms were included as fixed factors. Only nests that were first attempts for the season and for which female identity was known were included in the analysis. Because, in 2005, our newly established population was heavily biased towards SY females (Brasso and Cristol 2008), and because relatively few females (~60%) were captured in the first year of study, we chose to exclude data collected in 2005 from further analysis, electing instead to focus solely on 2006 and 2007, for which population age structure was more stable and a larger proportion of breeding females (>80%) had been captured.

Interactions with environmental conditions

To further explore how environmental conditions experienced during the breeding cycle might have mediated the relationship between mercury and reproduction, we performed a second series of GLMs integrating local climatic variables into the analyses. For each nest, we constructed a history consisting of the average maximum and minimum temperatures and precipitation encountered during (1) the 5 days prior to clutch initiation; (2) the first 10 days of incubation (beginning on day of clutch completion); (3) the first 7 days after hatching (nestling days 0–6); and (4) the next 7 days after hatching (nestling days 7–13). These time periods were chosen to reflect biologically meaningful periods in the nesting cycle. For example, in tree swallows, the period of rapid yolk synthesis is thought to be 4 or 5 days (Ardia et al. 2006a). Because subsequent egg formation takes approximately 24 h, a 5-day window prior to laying should enable us to examine the effects of weather conditions on egg quality. Additionally, we chose to examine only the first 10 days of incubation to ensure that all females would be included in a highly variable process that ordinarily lasts 11–14 days (Nooker et al. 2005). Finally, we divided the nestling period into two phases, each of which represents a different stage of nestling need. Nestling tree swallows have little ability to thermoregulate during the first 7–8 days of life (Robertson et al. 1992); thus, temperature might be expected to be a major factor influencing nestling survival during this period. As thermoregulatory abilities develop, nestlings enter a period of rapid growth, reaching a maximum mass of 22–24 g between days 12 and 14 (Robertson et al. 1992). During this stage, starvation, rather than hypo or hyperthermia, likely poses a greater threat to nestling survival.

In order to control for the potentially confounding effect of time of breeding on reproductive success (i.e., reproductive success tends to decline as season progresses; see Stutchbury and Robertson 1988), linear regressions of temperature and precipitation on date were conducted separately for each year. The standardized residuals of these regressions were used in all statistical tests, such that positive residuals represented conditions that were unseasonably warm or wet, and negative residuals represented conditions that were unusually cold or dry.

Because the reproductive success GLMs without weather information indicated no interactive effects of mercury with year or age, these two variables were not included in subsequent analyses. Each GLM included total number of fledglings produced as the response variable and site type as a fixed term. Additionally, a single weather variable (maximum temperature, minimum temperature, or precipitation for a given breeding stage) and its two-way interaction were included as fixed covariates. Thus, a total of 12 GLMs were performed, one for each possible breeding stage-weather variable combination. Of all of the reproductive endpoints measured (see above), number of fledglings was chosen to provide a single, holistic metric of reproductive success. For all analyses, data were pooled by date. Results are reported as mean ± SE.

Results

Mercury levels

There was a significant effect of site type on blood mercury concentration in breeding female tree swallows ($F = 523.49$, $p < 0.001$, df = 1,371). A post-hoc analysis revealed that blood mercury was significantly elevated on contaminated sites (Tukey HSD: $t = 22.88$, $p < 0.0001$), and was, on average, an order of magnitude higher than on reference sites (Table 1). This GLM also revealed a significant effect of year ($F = 30.02$, $p < 0.001$, df = 1,371), with mercury levels being significantly higher in 2006 than in 2007 (Tukey HSD: $t = 5.48$, $p < 0.0001$). This annual difference was only apparent, however, on contaminated sites; on reference sites, mercury levels were uniformly and similarly low in both years (year*site type interaction: $F = 36.79$, $p < 0.001$, df = 1,371; Fig. 1).

Effects on reproduction

Tree swallows breeding on contaminated sites initiated clutches approximately two days earlier than conspecifics breeding on reference sites ($F = 8.89$, $p = 0.003$, df = 1,360; Tukey HSD: $t = 2.98$, $p = 0.003$). Clutch size was similar across sites in both years of study ($F = 0.50$, $p = 0.48$, df = 1,375; Table 1). In fact, the only significant predictor of clutch size was age class; ASY females laid, on average, 0.61 more eggs than SY birds ($F = 29.75$, $p < 0.001$, df = 1,375; Tukey HSD: $t = 5.46$, $p < 0.0001$). Similarly, there was no effect of site type on egg volume ($F = 1.53$, $p = 0.22$, df = 1,83; Table 1). Hatching

Table 1 Average blood mercury and reproductive parameters for adult female Tree Swallows

	Contaminated	*n*	Reference	*n*	*p*
Blood mercury (μg/g)	3.03 ± 0.15	171	0.16 ± 0.005	207	<0.0001
Clutch initiation (days)	9 May ± 0.83	166	11 May ± 1.00	201	0.003
Clutch size	5.65 ± 0.08	170	5.71 ± 0.07	206	0.48
Egg volume (cm^3)[a]	1.77 ± 0.29	33	1.77 ± 0.22	57	0.22
% Eggs hatched	81 ± 2	155	91 ± 1	193	<0.0001
% Nestlings fledged[b]	80 ± 2	148	90 ± 2	191	0.0004
Number fledged	3.66 ± 0.15	154	4.66 ± 0.13	193	<0.0001

All nests (successful and unsuccessful) included in analyses unless stated otherwise. Data reported as mean ± SE. See text for details

[a] Eggs pooled by clutch

[b] Includes only nests in which ≥1 egg hatched

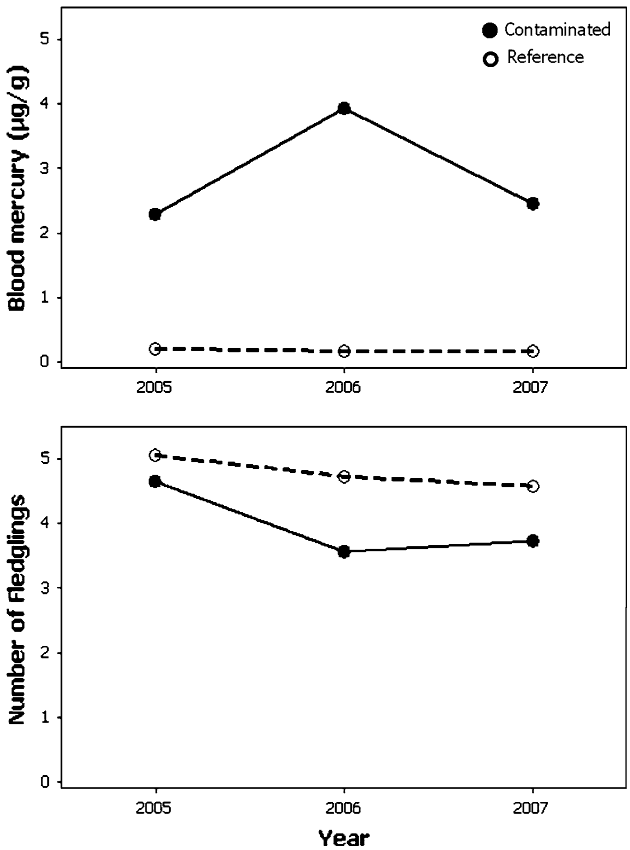

Fig. 1 Female blood mercury and number of fledglings produced in each year of study. Data from 2005 extracted from Brasso and Cristol (2008)

success, however, differed significantly between reference and contaminated sites ($F = 18.50$, $p < 0.001$, df = 1,341; Table 1). On reference sites, 91 ± 1% of eggs hatched; on contaminated sites, this percentage was reduced to 81 ± 2% (Tukey HSD: $t = 4.30$, $p < 0.0001$; Table 1). Although the proportion of eggs hatching also varied by female age ($F = 5.09$, $p = 0.03$, df = 1,341), neither the year*site type nor the age*site type terms were significant (year*site type: $F = 0.02$, $p = 0.88$; age*site type: $F = 2.09$, $p = 0.15$), indicating that the effect of mercury on hatching success was similar across both years and age classes. Finally, we observed a significant effect of site type on the proportion of nestlings that fledged ($F = 12.38$, $p < 0.001$, df = 1,332; Table 1). While females breeding on reference sites successfully fledged 90 ± 2% of their nestlings, only 80 ± 2% of nestlings hatched on contaminated sites survived to age of fledging (Tukey HSD: $t = 3.52$, $p = 0.0004$; Table 1). Together, the independent effects of site type on hatching success and fledging success amounted to a reduction in reproductive output of one fledgling per nest ($F = 19.84$, $p < 0.001$, df = 1,340; Tukey HSD: $t = 4.46$, $p < 0.0001$; Table 1).

Mediating role of weather

To examine whether the apparent effect of mercury on reproductive success (i.e., reduced number of fledglings) was modulated by environmental conditions experienced during the breeding cycle, we performed a second set of analyses in which we incorporated weather data from four different stages of nesting and examined their relationship to fledgling production (Fig. 2). As expected from the first analysis, all GLMs indicated a strong effect of site type on number of fledglings produced, with contaminated birds producing significantly fewer fledglings than conspecifics breeding on reference sites (all $p < 0.03$; Table 2).

Neither temperature nor precipitation during pre-laying or incubation exerted any detectable effect on number of fledglings produced (all $p > 0.07$; Table 2). However, during the early nestling period (nestling days 0–6), we observed a significant interaction between site type and daily maximum temperature ($F = 6.15$, $p = 0.02$, df = 1,105; Table 2). On contaminated sites, there was a negative relationship between daily maximum temperature and fledgling production (coef = −0.41 ± 0.18, $t = 2.32$, $p = 0.02$, df = 1,52). On reference sites, this relationship was positive (coef = 0.19 ± 0.17), though the association was weak ($t = 1.15$, $p = 0.26$, df = 1,53). A similar, albeit non-significant, pattern of interaction was observed when daily minimum temperature was substituted into the model ($F = 2.29$, $p = 0.13$, df = 1,105; Table 2). Thus, ambient temperatures interacted with mercury contamination such that high temperatures were detrimental to young nestlings on contaminated, but not on reference, sites. Precipitation during the early nestling period was not an important predictor of reproductive success in either contaminated or reference swallows (all $p > 0.42$; Table 2).

No interactions between mercury and any of the climatic variables were observed during the late nestling period (nestling days 7–13; Table 2). However, there was a strong, positive relationship between both daily maximum and minimum temperature and fledgling production that existed independent of the effects of mercury contamination (daily maximum temperature: $F = 5.64$, $p = 0.02$, coef = 0.30 ± 0.13, df = 1,105; daily minimum temperature: $F = 6.34$, $p = 0.01$, coef = 0.32 ± 0.13, df = 1,105; Table 2). Thus, as temperatures in the late nestling period increased, so did reproductive output on both contaminated and reference sites.

Discussion

Female tree swallows breeding along the mercury-contaminated South River in Virginia, USA were exposed to significantly elevated mercury. In fact, the levels of

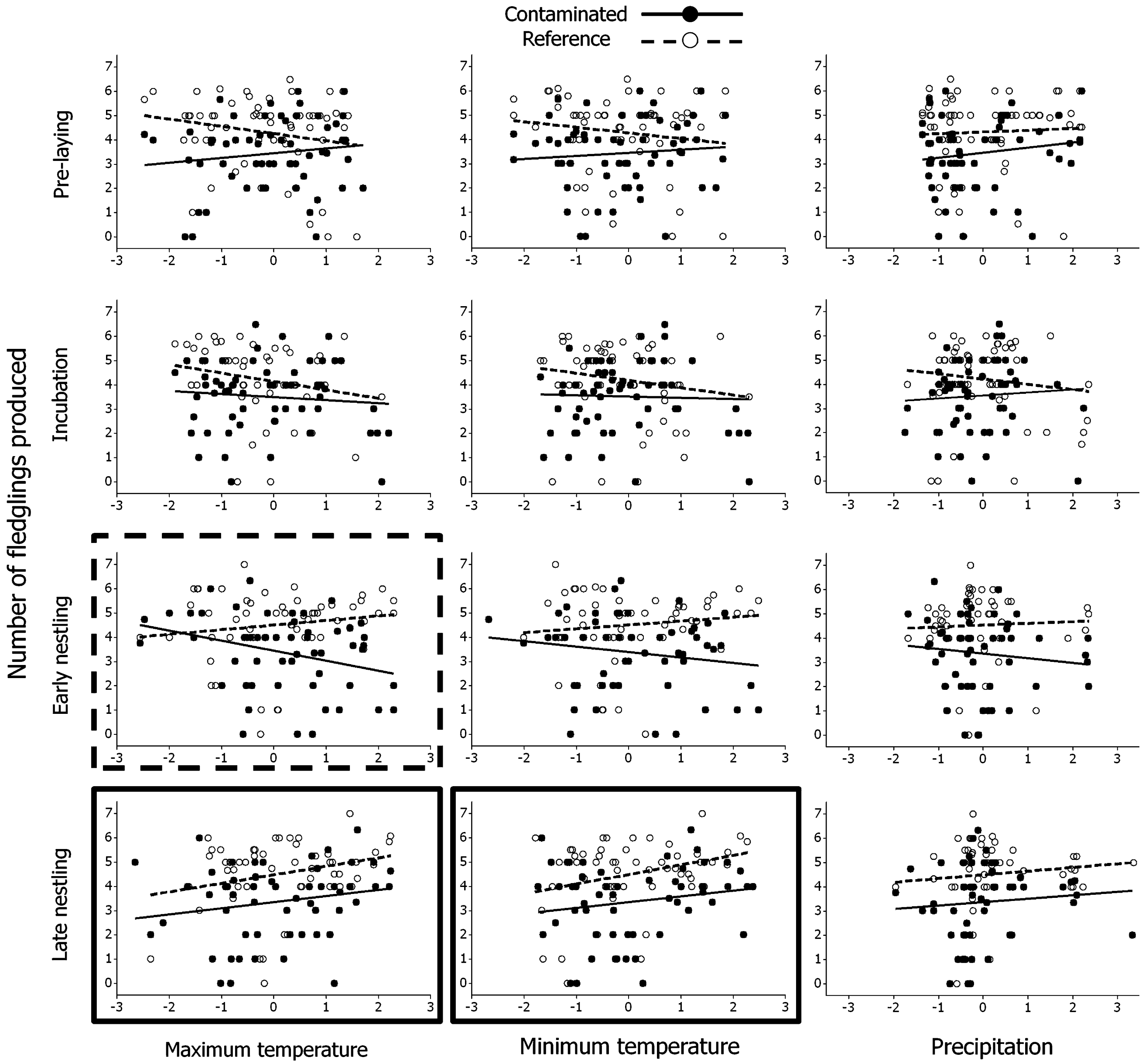

Fig. 2 Effects of weather and mercury on eventual number of fledglings produced. Three different weather variables (maximum temperature, minimum temperature, and precipitation) were evaluated during four different stages of breeding (pre-laying, incubation, early nestling, late nestling). Each weather variable was regressed on total fledgling production. Weather variables (horizontal axes) are coded as standardized residuals of seasonal averages, where negative values represent conditions that were either cooler or drier than average, and positive values represent conditions that were warmer or wetter than average. Models for which there was a significant effect of weather are surrounded by *solid boxes*. Significant site type*weather interactions are indicated by *dashed boxes*. Fledgling production was lower on contaminated sites (*solid lines*) in all cases

mercury found in this population rank among the highest ever reported in a free-living songbird (Brasso and Cristol 2008). Our data suggest that this high exposure was associated with lower reproductive success, as both hatching and fledging success were depressed on contaminated sites. As a result, swallows nesting on contaminated sites produced, on average, one less fledgling than those on reference sites, despite nesting 2 days earlier and producing eggs of normal volume and number.

Incorporating weather data into the analyses revealed a strong effect of daily maximum temperature during the early stage of the nestling period. Tree swallows, like all altricial songbirds, are born devoid of feathers or the capacity for thermoregulation, an ability that is likely not acquired until at least 8–9 days after hatching (Robertson et al. 1992). Thus, the task of temperature regulation during this critical period of nestling development falls largely on the adult female, usually in the form of brooding

Table 2 Summary of 12 models investigating the combined effects of mercury and weather on female Tree Swallow reproduction

Model[a]	Site type		Weather		Interaction		df	r^2
	F	*p*	F	*p*	F	*p*		
Egg T(max)	**8.65**	**0.004**	0.14	0.71	3.29	0.07	1,116	0.08
Egg T(min)	**8.71**	**0.004**	0.15	0.70	1.70	0.19	1,116	0.07
Egg P	**9.58**	**0.002**	1.11	0.29	0.27	0.61	1,116	0.07
Inc T(max)	**4.95**	**0.03**	2.64	0.11	0.57	0.45	1,111	0.05
Inc T(min)	**5.49**	**0.02**	1.32	0.25	0.63	0.43	1,111	0.04
Inc P	**5.82**	**0.02**	0.09	0.77	1.02	0.32	1,111	0.04
N1 T(max)	**13.98**	**<0.001**	0.85	0.36	**6.15**	**0.02**	1,105	0.16
N1 T(min)	**15.11**	**<0.001**	0.07	0.80	2.29	0.13	1,105	0.13
N1 P	**16.53**	**<0.001**	0.13	0.72	0.66	0.42	1,105	0.12
N2 T(max)	**15.89**	**<0.001**	**5.64**	**0.02**	0.14	0.71	1,105	0.15
N2 T(min)	**16.62**	**<0.001**	**6.34**	**0.01**	0.41	0.52	1,105	0.16
N2 P	**15.25**	**<0.001**	0.99	0.32	0.00	0.97	1,105	0.12

Each GLM was constructed as follows: number of fledglings produced ~ site type + weather variable + site type*weather variable, where 'site type' was either contaminated or reference, and 'weather variable' represented either maximum daily temperature, minimum daily temperature, or precipitation measured over the appropriate stage of breeding (pre-laying, incubation, early nestling, late nestling). For each model, all data were pooled by date. Significant terms are highlighted in bold

[a] For each model, *Egg* = weather recorded during pre-laying period, *Inc* = weather recorded during incubation, *N1* = weather recorded during early nestling period, *N2* = weather recorded during late nestling period. *T(max)* = daily maximum temperature, *T(min)* = daily minimum temperature, *P* = precipitation. For example, model, *Egg T(max)* represents the GLM: number of fledglings produced ~ site type + daily maximum temperature recorded during pre-laying period + two-way interaction. Other models coded accordingly

(Robertson et al. 1992). On contaminated sites, we observed a steady reduction in fledgling production as daily maximum temperatures in the early nestling period increased. In contrast, reference birds appeared to experience little, if any, variation in reproductive performance with temperature. Taken together, these patterns suggest that contaminated nestlings may have been particularly susceptible to stress during periods of unseasonable warmth. The fact that these trends disappeared during the late nestling period, at the peak of nestling growth, suggests that food was not a factor limiting reproductive success on contaminated sites.

High temperatures during the early nestling period might have been detrimental if they resulted in heat stress to contaminated nestlings. There are several mechanisms through which this could have occurred. First, it is possible that nest architecture differed such that contaminated nests were more insulated than nests on reference sites. If mercury altered nest-building behavior in such a way that the insulative properties of the nest were improved, i.e., through increased provisioning of feathers (Stephenson et al. 2009) or smaller nest cup size (Lombardo 1994), reproductive success might have been enhanced when temperatures were cool, but hindered when temperatures were warm. It would not be surprising to find that hormonally-mediated behavioral processes are affected by mercury. Mercury can act as an endocrine disrupter (Wada et al. 2009; Frederick and Jayasena 2010), and it is likely that nest-building behavior is hormonally-mediated in this species. In fact, nest-building behavior has been shown to be altered in tree swallows that were exposed to polychlorinated biphenyls, a well-documented endocrine disrupter (McCarty and Secord 1999).

Another possibility is that females nesting on contaminated sites exhibited irregular brooding behavior, such that they over-brooded their nestlings, or brooded at inappropriate times. As with nest-building, there is likely a strong endocrine basis for broodiness (Angelier and Chastel 2010), and the pathways controlling this suite of behaviors might have been disrupted by mercury. However, it is not immediately clear why brooding would be affected when incubation appeared effective, given that the underlying physiological processes appear similar, and that the risk of hyperthermia to eggs may be high (Ardia et al. 2006b).

Finally, it is possible that susceptibility to heat stress among contaminated nestlings arose as a direct result of altered nestling physiology, rather than as an indirect effect of altered parental behavior. Previous research on tree swallow nestlings raised on the South River has documented suppression of both adrenocortical response and thyroid hormone levels (Wada et al. 2009). Given that both endocrine axes are at least peripherally involved in the development of thermoregulation (Debonne et al. 2008), it seems quite plausible that their disruption might result in a reduced ability to regulate temperature.

Even mild hyperthermia could have hindered the survival of young nestlings on contaminated sites. In the absence of thermoregulatory capabilities, the metabolism of young swallows tends to rise and fall passively in tandem with the thermal gradient to which the nestlings are exposed (McCarty 1995), Thus, at low temperatures, young nestlings expend very little energy; indeed, this has even been posited as an adaptive strategy for conserving energy during periods of food stress (McCarty 1995). As temperatures rise, so does the metabolism of poikilothermic nestlings (McCarty 1995). It is easy to hypothesize a scenario wherein a chronically high body temperature inadvertently leads to depletion of resources and ultimately, starvation. Such an effect could be exacerbated if rates of parasitism are also higher on contaminated sites (see Gentes et al. 2007).

Variation in temperature and precipitation did not help explain observed differences in hatching success between contaminated and reference birds. However, mercury is known to exert direct embryotoxic effects in a number of bird species, including tree swallows (Heinz et al. 2009). Thus, it is possible that contaminated females deposited large enough quantities of mercury into their eggs to reduce hatching success appreciably, irrespective of weather conditions encountered prior to laying or during incubation. In fact, elevated egg mercury levels have already been described in this population (Brasso et al. 2010).

It is important to consider that a number of differences exist between our study and an earlier one conducted by Brasso and Cristol (2008). Most notably, the latter reported a reduction in fledgling production on contaminated sites, but only among SY females and only in one breeding season (2006) of a two-year study (2005–2006). To account for these results, the authors suggested that a severe drought in 2006 led to increases in both mercury burden and physiological stress. The combination of these stressors may have resulted in a perfect storm whereby the youngest and least experienced breeders in the population were unable to cope (Brasso and Cristol 2008). In support of this hypothesis, the authors observed that SY females on contaminated sites had laid significantly smaller eggs than their reference counterparts, suggesting one possible mechanism through which their reproductive success might have been compromised (Brasso and Cristol 2008). In our study, we re-examined this hypothesis using a larger data set collected from the same population after it had been established for 2–3 breeding seasons. We found that reproductive impairments were more pervasive than previously reported (Brasso and Cristol 2008), with mercury affecting hatching, as well as fledging, success across both female age classes, and in both years of study (2006 and 2007).

It is instructive to consider why the differences between these two studies occurred and how they may be reconciled to provide an accurate portrait of mercury's effects on reproductive success in this population:

(1) Sample size: It is possible that differences resulted from variation in sample sizes between the two studies. Sample sizes in the present study were at least twice as large as in Brasso and Cristol (2008). Admittedly, a lack of statistical power is unlikely to account for all of the observed differences—a visual examination of mean reproductive rates reported in Brasso and Cristol (2008) suggests that no reduction in reproductive success occurred in 2005. However, low sample size may help to explain the earlier finding that smaller eggs were laid by SY females on contaminated sites, as Brasso and Cristol (2008) measured only six clutches. For the present study, 16 clutches were examined and no such difference was found.

(2) Cumulative effect of mercury: The effects of mercury on reproduction may increase in each subsequent breeding season. This could explain the observation that reproductive abnormalities were largely absent in 2005, the first year in which tree swallows were exposed to mercury at this site. Examining each season in isolation suggests support for this hypothesis, as the apparent extent of reproductive effects in contaminated birds increased from 2005 to 2006 to 2007; however, detailed multi-year studies of individual females will be required before any conclusion can be drawn about cumulative effects.

(3) Annual differences in mercury exposure: Average blood mercury level was significantly higher in 2006 than in 2005, and this was hypothesized as a cause for worse reproductive success in 2006 (Brasso and Cristol 2008). However, we observed no obvious correspondence between average annual mercury level and reproductive success—mercury levels in 2007 dropped back to those reported in 2005, yet fledgling production remained depressed in contaminated birds (Fig. 1). Clearly, the relationship between mercury exposure and reproductive success is more complex, a fact further highlighted by the lack of a strong dose–response among individual females (see Brasso and Cristol 2008).

(4) Annual differences in weather: One of the most intriguing insights gained from our analysis of weather-mercury interactions was that females responded differently to daily maximum temperatures during the early nestling period depending on whether they were breeding on contaminated or reference sites. In general, it appears that unusually warm temperatures in the first week after hatching seemed to accentuate differences in reproductive success, while cooler temperatures tended to mask them. If such a pattern exists within a season, then it also stands to reason that interannual differences in temperature might exert a similar effect. In fact, mean daily maximum temperatures during the early nestling period were lower in 2005 (23.0°C), when no effect of mercury was detected, than in either 2006 (24.1°C) or 2007 (26.8°C; One-way ANOVA: $F = 60.94$, $p < 0.001$, df = 2,391), consistent with the hypothesis that reproductive effects of mercury are larger when young nestlings face heat stress.

It is also worth noting that we detected no effect of variation in precipitation on the relationship between mercury and reproduction, in contrast to the putative effects of drought previously reported for this population (Brasso and Cristol 2008; see also Hill et al. 2008). However, the amount of variation in precipitation was limited during this study, allowing the possibility that we would have detected mediating effects if more birds had nested during extreme heavy rain conditions. In addition to the amount of precipitation, it is likely that the duration of rainy weather and its coincidence with cold temperatures is important, because of the depressive effect on flying insects. Future studies should examine precipitation in more detail than was attempted here.

Our data do not allow us to link mercury contamination unequivocally to specific mechanisms of reproductive impairment. However, the analyses presented here do offer potential insight into the underlying causes of the deficits in hatching and fledging success that we observed. In particular, young nestlings appear most susceptible to the effects of mercury during periods of heat stress. Knowledge of conditions under which reproductive effects are manifest may enable wildlife managers to better predict which populations may be at the greatest risk of suffering impairments. If, for example, contaminant effects on nestlings are manifest only during extreme temperature events, sites at the edges of the range might be given priority for remediation. In addition, when undertaking risk assessment for the effects of contaminants during predicted warmer climates in the future, it will be important to take the key finding of the present study into account—contaminants that cause little or no injury under moderate temperatures might exert greater effects at higher ambient temperatures.

Finally, although we observed an overall effect of mercury contamination on both hatching and fledging success, the magnitude of this difference was susceptible to variation in ambient temperature. Had we performed this study during average conditions, or during extreme weather at either end of the temperature spectrum, we might have observed an effect of mercury that was larger or smaller than that observed here. This raises the possibility that contaminant effects may often be masked by unusually favorable (or poor) weather conditions (see also Custer et al. 2003; Gentes et al. 2006; Hill et al. 2008). Likewise, studies reporting a large effect of mercury on individual fitness metrics may, in some cases, simply have observed subjects under unusually discriminating conditions. This is especially worth considering when interpreting the results of laboratory studies, in which most environmental variation has been intentionally removed. A better understanding is required of the mechanisms by which mercury impacts avian reproduction, but this study shows that progress can be made by examining the interactive effects of weather conditions.

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