

## Assessing avian incubation behavior in response to environmental pollution with temperature-humidity loggers

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The negative impact of environmental pollution on avian physiology and breeding success is well documented. However, pollution-related behavioral changes during reproduction remain underexplored, despite behavior often being one of the earliest indicators of environmental disturbances and having significant life-history consequences. For example, altered food availability in a polluted environment could potentially perturb the incubation behavior of income breeders. These birds typically alternate between staying in the nest and heating eggs (on-bout) and taking foraging trips (off-bout). In this two-year study (2020 and 2022), we investigated how the incubation behavior of an insectivorous passerine, the pied flycatcher (*Ficedula hypoleuca*), varied with environmental pollution levels around a Cu-Ni smelter. Additionally, we compared two different metrics – temperature and humidity within the nest – to evaluate their use as indicators of incubation rhythm. We found that temperature- and humidity-based incubation rhythm parameters correlated, but those based on humidity matched better the true incubation behavior documented by simultaneous video recording. This was because the humidity curve showed a more immediate and intensive response to the female's incubation behavior. Birds in the polluted area took slightly more (11%) but shorter (11%) off-bouts, possibly reflecting smaller energetic constraints or better food availability in the polluted area. However, we found no difference in total incubation intensity between polluted and control areas, with *F. hypoleuca* females incubating their eggs 75% of the daytime in both environments. Hence, incubating females in the polluted area did not allocate more time for gathering their energy reserves than the birds in the control area, and there was also no difference in the hatching success. Our study is the first to use humidity variation to record incubation rhythm, and our results indicate that measuring humidity inside the nest is a promising technique to test and develop further. For example, further studies are needed to test if this method would work in different types of nests. From an environmental protection standpoint, our results also contribute valuable insights to the relatively limited information on pollution-related behavioral changes.



## 1. Introduction

Environmental pollution, such as industrial emissions, can alter the habitat and the quality of food resources available to birds, potentially leading to both direct and indirect effects on their reproduction (Morrison 1986, Furness & Greenwood 1993, Eeva *et al.* 2005, Belskii & Belskaya 2013). The influence of industrial emissions becomes particularly apparent during breeding, since *e.g.* metal emissions and acidifying compounds can affect the vegetation and invertebrate numbers, reducing the amount of birds' prey during the period when food consumption is at its highest (Belskii *et al.* 1995, Eeva *et al.* 1997). So far, studies have mainly focused on reproductive output or physiological effects caused by pollution, while there is relatively little information about the potential impacts of environmental pollution on birds' behavior during reproduction, which could partly explain the links between pollution and reproductive success (Sanderfoot & Holloway 2017). For example, behavioral changes could reveal secondary effects of pollution that might not be immediately apparent through physiological measurements but could be important early warning signals from the conservation point of view. One reason for the small amount of behavioral research is that behavioral studies tend to require extensive field effort. However, recent technological advances and the miniaturization of data loggers have facilitated some behavioral studies, such as the measurement of birds' incubation behavior, which is a critical aspect of parental care in birds and can affect the development of embryos (Smith *et al.* 2015, Hope *et al.* 2022).

Almost all birds incubate their eggs by warming them to reach the required temperature with the heat being transferred via an abdominal skin area called the brood patch. This extra cost, in terms of heat loss and time, may be energetically demanding for the parents (*e.g.* Bryan & Bryant 1999, but see Ilmonen *et al.* 2002, Nord *et al.* 2010). In order to compensate for the increased energy requirements during this period, incubating birds need to allocate more time for foraging. Depending on the species, the incubating bird may either forage itself or the other parent may

partly or fully feed the incubating partner (Koski *et al.* 2020). Incubation intensity largely determines the early developmental trajectory of bird embryos (DuRant *et al.* 2013). Ectothermic bird embryos need an optimal developmental temperature (36–38°C; Tieleman *et al.* 2004), which is not often matched by the environment (Camfield & Martin 2009). This challenge for birds is accentuated when only one parent incubates (Hu *et al.* 2024). For instance, over 60% of Passeriformes families demonstrate female-only incubation (Deeming 2002). Consequently, uniparental incubation patterns reflect a trade-off between self-maintenance (foraging) and the temperature requirement of the embryos (Cooper & Voss 2013). The alternating periods, where females leave the clutch to obtain food (off-bout) and return to warm the eggs (on-bout), are called the incubation rhythm (von Haartman 1958).

Industrial emissions can restrict food availability by disrupting food chains. This can occur through the accumulation of toxic substances in the environment, which could harm or reduce populations of key species within the ecosystem, ultimately leading to a decrease in the food supply for higher trophic levels. This may pose a problem not only for growing nestlings but also for incubating parents (Eeva *et al.* 1997). In normal conditions, birds that take many off-bouts also tend to take shorter off-bouts to keep incubation temperatures more stable (Cooper & Voss 2013). However, in low-quality territories, birds are more likely to take more foraging trips and have longer off-bouts and shorter on-bouts, which increases egg temperature variation (DuRant *et al.* 2013, Koski *et al.* 2020). Polluted sites may represent such low-quality territories, where vegetation and invertebrates suffer from pollution exposure with negative consequences to insectivorous birds (Kiikkilä 2003, Eeva *et al.* 2005).

Point sources of pollution provide a good opportunity to test behavioral effects between polluted and relatively unpolluted environments. A long-term follow-up study around a Finnish copper-nickel smelter, a point source of metal pollution, has revealed pollution-related reductions in breeding parameters and physiological changes in some small insectivorous passerines, such as the pied flycatcher (*Ficedula hypoleuca*)

(Eeva & Lehikoinen 1995, Espin *et al.* 2017). In this and other similar environments, the composition and nutritive quality of prey species varies depending on the level of pollution (Eeva *et al.* 2005, Belskii & Belskaya 2013). To study the impact of pollution on birds' breeding behavior, we explored whether the incubation rhythm and intensity of *F. hypoleuca* females differ between the polluted sites close to the smelter and relatively unpolluted control sites farther away. We also took into account several other parameters that could potentially influence the incubation rhythm, such as ambient temperature, time of day, and phase of incubation. In addition, we tested a potential new method to study birds' incubation behavior: incubation rhythm is often studied by measuring incubation-dependent temperature variation in the nest, but here we test if humidity variation could be used for the same purpose. Testing another method was motivated by our prior pilot study (T. Eeva, unpublished), which suggested that the humidity curve may even show a faster and stronger response than the temperature curve for tracking the incubation rhythm.

We expected that the indirect effects of heavy metal pollution, such as habitat deterioration and related scarcity of suitable food, which have been documented in our study area (Eeva *et al.* 2005), could affect birds' incubation rhythm. In particular, we hypothesized that, in the polluted areas, birds might take more and/or longer off-bouts to find enough food, due to a trade-off between fueling and incubation intensity. Furthermore, we expected that measuring humidity could provide a novel means to measure incubation rhythm and potentially provide better-quality data than temperature.

## 2. Material and methods

### 2.1. Study area

One of the main sources of local air pollutants in Finland is a factory complex located in the center of Harjavalta town (61°20'N, 22°10'E). Arsenic, copper, nickel, lead, and zinc are common pollutants in the area (Kiikkilä 2003).

Elevated heavy metal concentrations occur in the soil and biota of the polluted area but decrease exponentially with increasing distance to the smelter, approaching background levels at sites further than five kilometers from it (Eeva & Lehikoinen 1996). Based on this information, nine study sites were established along an air pollution gradient in three main directions (SW, SE, and NW) around the town (Fig. 1). Five sites were classified as "controls" (>5 km from the factory complex) and four as "polluted" (<2.5 km from the factory complex) and these distance classes are used in the statistical analyses. Each site contains between 20–60 wooden nest boxes attached to a tree. The design of nest boxes has been described by Lambrechts *et al.* (2010). Special attention was paid to select study sites which represent similar habitat type, *i.e.* relatively barren forests dominated by Scotch pine (*Pinus sylvestris*), which forms mixed stands with spruce (*Picea abies*), and birches (*Betula spp.*). However, vegetation near the smelter has suffered from the long-term pollution and *e.g.* the ground layer vegetation cover is patchy or lacking at more heavily polluted locations (Kiikkilä 2003).

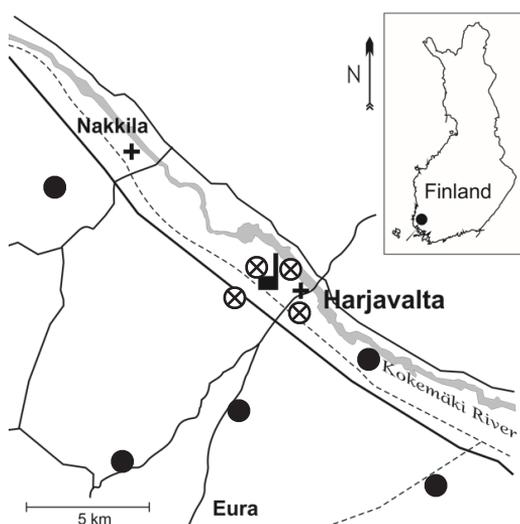


Fig. 1. Location of nine study sites, with the factory complex in the center (black dots = control site, crossed dots = polluted site).

## 2.2. Study species

Like many other long-term population studies in birds, pollution-related studies have mainly focused on hole-breeding birds such as *F. hypoleuca* (Çelik *et al.* 2021). This migratory and insectivorous species, which typically lays only one clutch per season, breeds in a wide area in northern Eurasia (Cramp 1988). The female normally lays one egg per day until having a clutch size of 5 to 8 eggs, and, after laying the last egg, starts the incubation period of *ca.* two weeks (Lundberg & Alatalo 1992). Incubating females forage themselves, but the male also feeds the female, and high contribution by a male may increase the female's body mass and nest attendance, although this is condition-dependent (von Haartman 1958, Lifjeld & Slagsvold 1986, Cantarero *et al.* 2014). In our study, however, the male's feeding activity could not be estimated. Typical prey includes spiders, caterpillars, and winged insects (*e.g.* moths *Lepidoptera*, beetles *Coleoptera*, and sawflies *Symphyla*, depending on the environment) (Eeva *et al.* 2005). This species also readily accumulates heavy metals, and a few weeks after the arrival from their wintering grounds in Africa, female birds show increased tissue concentrations of metals in polluted locations (Eeva & Lehikoinen 2004, Berglund *et al.* 2011).

## 2.3. Measurements of incubation rhythm

In 2020 and 2022, all nest boxes were inspected at least once per week (starting at the end of April) to record basic breeding data such as timing of breeding, clutch size, and number of hatchlings. When we encountered a nest with warm eggs (*i.e.* a female had started to incubate), a high-resolution hygrochron iButtons® DS1923-F5 (Maxim Integrated Products 2020) were placed inside the nest materials in the summer of 2020 (n=28 nests) to measure incubation rhythm by recording temperature and relative humidity. Measurements were not taken on rainy days. Loggers were placed underneath the eggs within the nest lining layer, since preliminary studies have shown that eggs can break during the incubation period if loggers are placed among them (Schöll *et al.* 2020). A

plastic key ring mount (iButton, DS9093AB+) was attached to each logger to avoid displacement of the small loggers in the nest. Humidity loggers were not available in 2022, and we used temperature iButtons DS1922-F5 instead (n=44 nests). The loggers were programmed to measure temperature (°C) and relative humidity (% RH) at intervals of 30 seconds with a resolution of 0.5 °C (2020) or 0.063 °C (2022) for temperature and 0.6 % RH (2020) for humidity. For logistic reasons, the logger installation time varied from 8:25 am to 15:49 pm, and the recording period length varied from 4 h 45 min to 10 h 25 min (mean ± SD: 7.8 ± 1.4 h). For the same reason, the estimated phase of incubation varied from incubation day 1 (= 1<sup>st</sup> incubation day) to day 13 (mean ± SD: 7.3 ± 2.6 days). Because clutch size is known to affect the egg cooling rate, which is faster in smaller clutches (Boulton & Cassey 2012), we standardized the sampling by selecting focal nests of primarily 6 or 7 eggs, although two nests with smaller clutches were measured when suitable nests were not available (removing those nests from our analyses did not change qualitatively the results). Female birds were also trapped for ringing and age determination, which was based on their plumage characteristics (Svensson 1992). However, age-related differences in plumage are relatively small, and we have estimated that <5% of age determination can be erroneous (Eeva *et al.* 2018). Females were usually trapped a few days before the incubation rhythm measurements, but never on the same day right before the measurements, to avoid disturbance.

Another logger was placed on the outer back wall of the nest box, recording the ambient temperature (2020 and 2022) and humidity (2020 only) every 30 seconds. This was done because, in the data analysis phase, the ambient temperature and humidity were used as background values to take into account the ambient changes during the measurement period. Furthermore, ambient temperature was used in statistical models as an explanatory variable. Finally, we verified the iButton measurements inside one nest by simultaneously video recording the entrance of the nest box, allowing a comparison of visually documented incubation rhythm with the iButton data (see also Bueno-Enciso *et al.* 2017). The observations and measurements were made under

the licenses from the Finnish Centre for Economic Development, Transport and the Environment (VARELY/3622/2017, VARELY/6817/2021).

## 2.4. Data handling and statistics

### 2.4.1. Calculation of incubation rhythm parameters

We calculated the off-nest bout frequency (variable BPerH; number of off-bouts/h), mean duration of on-bouts (MOnDur; min), mean duration of off-bouts (MOffDur; min), total on-bout duration (TOnDur; min/h; hereafter “incubation intensity”) and mean ambient air temperature (°C) during the measurement period by using NestIQ v.0.2.5 software (Hawkins & DuRant 2020). Following the previously used parameters for investigating incubation behavior of *F. hypoleuca*, we set the detection threshold to a 1°C decrease in temperature and a 1% decrease in humidity lasting at least 4 minutes (Koski *et al.* 2020). Note that on- and off-bout duration times are not opposite or mutually constrained measures, and they can vary independently. Considering the possible disturbance to the natural incubation rhythm because of installing the logger in the nest, we excluded data of the first on-bout/off-bout incubation cycle after visiting the nests. Five low-quality measurements were discarded from further analyses (2020 n=3, 2022 n=2). A possible reason for low-quality measurements was placing the logger too deep in the nest material, and in one case, logger failure. The final sample number was 67 measurement periods (25 nests in 2020 and 42 nests in 2022). Age determination was missing for three females, decreasing the sample number in statistical tests where age was included. Each nest was measured only once. Statistical tests were performed in SAS 9.4 software (SAS Institute Inc. 2013).

### 2.4.2. Comparison of temperature- vs. humidity-based measures (2020)

First, we tested correlations among the measures produced by two different measurement techniques in the dataset of 2020. Because some of the variables (temperature-based BPerH and MOnDur, humidity-based MOnDur) showed

deviations from normal distribution, we used Spearman’s rank correlation tests. Second, we compared temperature-based and humidity-based estimates with pairwise t-tests. Normality of distributions was confirmed by Kolmogorov-Smirnov tests. Finally, we inspected one video recording of a pied flycatcher nest by manually determining all on- and off-bout durations and the number of bouts. From this information, we explored temporal match of temperature- and humidity-based data with video-based observations.

### 2.4.3. Variation in temperature-based incubation rhythm parameters (2020 and 2022)

Variation in the four incubation rhythm parameters was studied with linear (LM) and generalized linear (GLM) models. For off-bout frequency, we used GLM with Poisson error distribution, while the other response variables were tested with LM with normal error distribution. Furthermore, we tested differences in hatchability of eggs between polluted and control areas with GLM, where the hatching probability of eggs was modeled with binary error distribution (using events/trial syntax of SAS). Model fits were confirmed by checking the overdispersion parameter (Pearson Chi-square/df) for the GLM models and normality of model residuals for LMs. The main explanatory factors were area (polluted vs. control) and year (2020 and 2022). Their interaction effect was non-significant in all models, and we did not include the interaction term in the final analyses. Instead, we tested other factors which may affect avian incubation. First, the ambient temperature outside the nest box has been shown to affect the incubation intensity, which was higher in cool conditions (Conway & Martin 2000, Arct *et al.* 2022). Second, the age of the female (two categories: young vs. old) was considered as a potential covariate of foraging efficiency (Joyce *et al.* 2001, Cauchard *et al.* 2021). Finally, time of measurement (at start) and phase of incubation (estimated number of days incubated) were included in the models as possible confounding factors (Cooper & Voss 2013, Hope *et al.* 2022). Although the length of measurement periods also varied, we did not include it in the statistical models because there was no *a priori* expectation that it would

affect the incubation rhythm on the top of the effect of time of day. Since the exact day of the start of incubation was not always known, we calculated this estimate from the laying date and egg number, with the defaults of one egg laid per day and incubation started after the last egg, which is the norm in this species although *e.g.* laying gaps may cause extra variation in this estimate (Koski *et al.* 2020, Gładalski *et al.* 2020). Laying date was also first considered as a covariate but since it correlated significantly with ambient temperature ( $r=0.49$ ,  $n=67$ ,  $p<0.0001$ ), we did not include it in the models to avoid collinearity between explanatory variables. The categorical variables area and year were retained in all models, while non-significant covariates were removed or left in the model by using model AIC value as a criterion (Burnham *et al.* 2011).

### 3. Results

#### 3.1. Comparison of humidity- vs. temperature-based data (2020 dataset)

Off-bout frequencies (BPerH) based on temperature and humidity correlated positively ( $n=25$ ,  $\rho=0.69$ ,  $p<0.0001$ ). The temperature-based data showed lower off-bout frequencies ( $\mu \pm SD = 2.78 \pm 0.50$  bout/hour) than humidity-based data ( $\mu \pm SD = 3.49 \pm 0.78$  bout/hour) (paired t-test,  $df=24$ ,  $t=-5.87$ ,  $p<0.0001$ ; Fig. 2a).

The mean off-bout duration (MOffDur) showed a significant positive correlation ( $n=25$ ,  $\rho=0.55$ ,  $p=0.0041$ ) between temperature- and humidity-based values. Temperature-based data showed 21% longer off-bout duration times ( $\mu \pm SD = 5.06 \pm 0.59$  min) than humidity-based data ( $\mu \pm SD = 4.19 \pm 0.75$  min) (paired t-test,  $df=24$ ,  $t=6.45$ ,  $p<0.0001$ ; Fig. 2b).

The two measures of mean on-bout duration (MOnDur) correlated positively ( $n=25$ ,  $\rho=0.66$ ,  $p=0.0003$ ). Mean on-bout duration based on temperature data ( $\mu \pm SD = 15.7 \pm 4.10$  min) was higher than that based on humidity data ( $\mu \pm SD = 12.6 \pm 4.66$  min) (paired t-test,  $df=24$ ,  $t=4.79$ ,  $p<0.0001$ ; Fig. 2c), indicating that values based on temperature and humidity are well correlated but temperature produces 25% higher values for the nest attendance time. Mean off-bout duration

did not correlate with the mean on-bout duration for either method ( $p>0.6$  in both), indicating that these two measures varied independently.

Incubation intensity (TOnDur) showed a significant positive correlation ( $n=25$ ,  $\rho=0.57$ ,  $p=0.0027$ ) between temperature- and humidity-based values. There was no significant difference between temperature-based ( $\mu \pm SD = 45.9 \pm 3.20$  min/h) and humidity-based incubation intensities ( $\mu \pm SD = 45.4 \pm 3.67$  min/h) (paired t-test,  $df=24$ ,  $t=0.92$ ,  $p=0.37$ ; Fig. 2d). The overall mean temperature below the nest cup was  $27.0 \pm 2.36$  °C, and mean relative humidity  $38.4 \pm 6.98\%$  ( $n=25$  nests).

#### 3.2. Comparison of iButton data with video-based values (2022)

Temporal variation in temperature- and humidity are generally in good agreement with simultaneous video-based observations of the incubation rhythm (Fig. 3). However, differences between nest entry and exit times given by NestIQ and those revealed by direct video observations indicated that humidity reacted much faster than temperature to female movements: mean delay after entry was 29.5 s for humidity and 107.9 s for temperature. In other words, humidity starts to increase soon after the bird enters the nest, while temperature shows a longer lag (Fig. 3). At the moment of exit, both lags are shorter than at entry, but humidity response is still much faster (2.07 s) compared to temperature (77.3 s).

Finally, we compared how well the logger-based incubation rhythm parameters given by NestIQ match the values measured from the video recording (Table 1). The bout number estimates were similar, except that humidity showed one more on-bout observation (the last one shown in Fig. 3). Humidity- and temperature-based on-bout duration estimates were slightly shorter while off-bout duration estimates were longer, especially for the temperature (Table 1). Taken together, humidity-based estimates of bout numbers reflect well the temperature-based estimates but, since humidity curve tracks faster the true incubation rhythm, the humidity-based off-bout durations match better with true values and result in a less biased estimate.

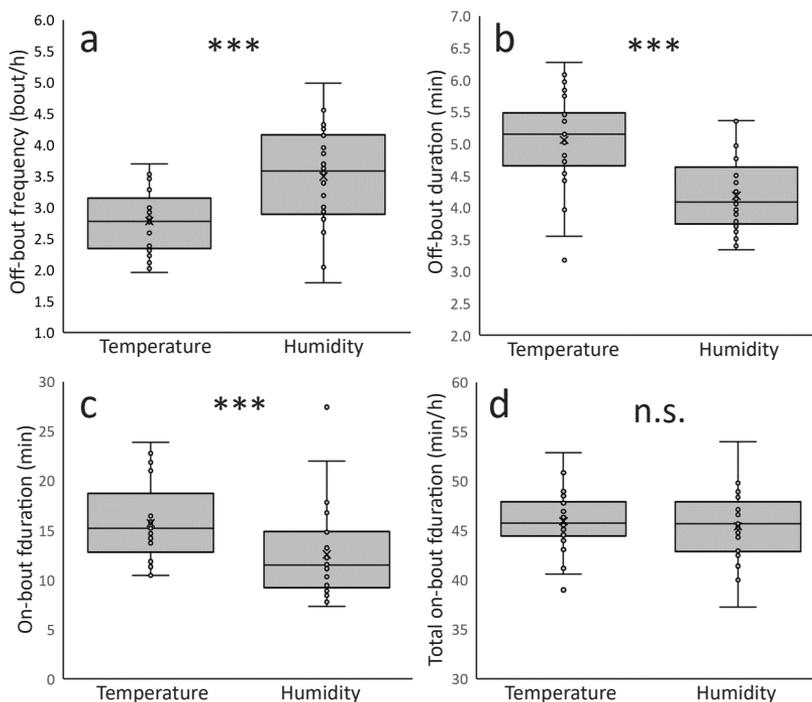


Fig. 2. Boxplots (min, max, median, 1<sup>st</sup> and 3<sup>rd</sup> quartiles, mean shown by ×) of four incubation parameters from the 2020 dataset based on temperature and humidity measurements. Mean off-bout frequency (bout/hour; a), mean off-bout duration (min; b), mean on-bout duration (min; c) and incubation intensity (min/h; d). Pairwise T-tests for between-group differences (n=25 nests): n.s. = not significant, \*\*\* = p<0.001.

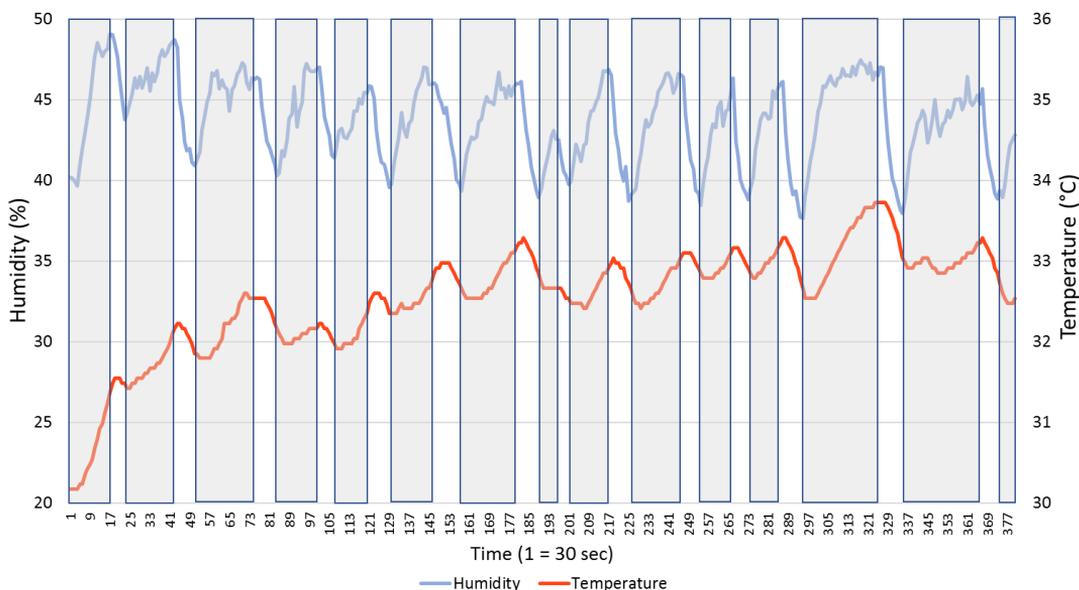


Fig. 3. Graphs of relative humidity and temperature recorded by iButton superimposed with on-bout periods (grey frames) obtained by video recording indicating that the female was inside the nest box. One unit on the x-axis equals 30 seconds (video length 3.1 h). Blue (upper line) = humidity, red (lower line) = temperature.

Since the NestIQ software detects bout transitions based on changes in temperature and humidity, with larger fluctuations being more readily detected, we calculated the coefficients of variation (CV) for both measures in each nest to assess their variability. Humidity exhibited a CV 4.4 times higher than that of temperature (CV for temperature = 3.3%, CV for humidity = 14.5%; paired t-test,  $df=24$ ,  $t=-12.6$ ,  $p<0.0001$ ), indicating significantly greater fluctuations in the humidity data.

### 3.3. Pollution effects based on temperature data (2020 and 2022)

The median values of the temperature-based incubation rhythm parameters for polluted and control areas are shown in Fig. 4. Off-bout frequency was 11.0% higher and off-bout duration 10.9% shorter in the polluted area, while there were no significant differences in the other parameters between the areas (Table 2, Fig. 4). None of the parameters were dependent

Table 1. Four incubation parameters compared between three measurement methods: video recording, humidity logger and temperature logger (mean  $\pm$  SD). Percentages in parenthesis were obtained comparing humidity- or temperature-based values with ones obtained from the video recording.

	Off-bout frequency	Mean off-bout duration (min)	On-bout frequency	Mean on-bout duration (min)
Video	14	3.05 $\pm$ 0.83	15	9.77 $\pm$ 3.76
Humidity	14	3.21 $\pm$ 0.97 (+5%)	15	8.67 $\pm$ 3.37 (-13%)
Temperature	14	3.96 $\pm$ 1.85 (+30%)	14	8.57 $\pm$ 4.40 (-14%)

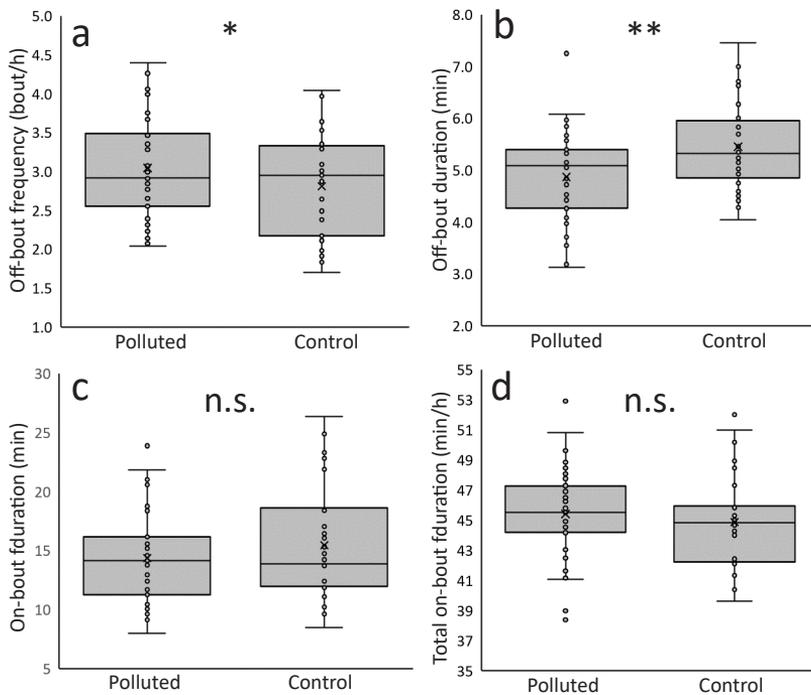


Fig. 4. Boxplots of four incubation parameters in polluted and control areas. Seasons 2020 and 2022 combined ( $n=67$  nests). Mean off-bout frequency (bout/hour; a), mean off-bout duration (min; b), mean on-bout duration (min; c) and incubation intensity (min/h; d). Linear model results for differences among areas: n.s. = not significant, \* =  $p<0.05$ , \*\* =  $p<0.01$  (see Table 2).

on the year of measurement or female age (Table 2). The mean off-bout duration increased, whereas on-bout duration and incubation intensity decreased with increasing ambient temperature (Table 2, Fig. 5). On-bout duration and incubation intensity slightly increased with time of day (Table 2). Furthermore, the off-bout frequency increased but the on-bout duration and incubation intensity decreased with advancing incubation phase (Table 2). The overall mean incubation intensity was  $45.2 \pm 3.12$  min/h ( $n=67$ ). Hatching probability of eggs did not significantly differ between the areas (polluted 0.92; control 0.87; GLM:  $F_{1,65}=0.89$ ,  $p=0.35$ ). Ambient temperatures at the time of measurements increased on average  $0.53$  °C per day (LM:  $F_{1,63}=37.2$ ,

$p<0.0001$ ) and were  $1.5^\circ\text{C}$  higher in 2020 than in 2022 (LM:  $F_{1,63}=6.48$ ,  $p=0.013$ ) but did not differ significantly between the areas (polluted:  $20.0$  °C; control:  $19.6$  °C).

## 4. Discussion

### 4.1. Humidity vs. temperature as measures of incubation rhythm

Our study suggests that relative humidity is a good alternative to temperature for measuring incubation rhythms. Temperature-based data showed lower off-bout frequencies and longer off- and on-bout duration times than humidity-based

Table 2. Linear models<sup>1</sup> to explain variation in four incubation rhythm related parameters of *Ficedula hypoleuca*: off-bout frequency (BPerH), mean off-bout duration (MOffDur), mean on-bout duration (MOnDur) and incubation intensity (TOnDur). N=67 nests.

Source of variation	BPerH		MOffDur		MOnDur		TOnDur	
	Est.	F <sub>df</sub>	Est.	F <sub>df</sub>	Est.	F <sub>df</sub>	Est.	F <sub>df</sub>
Intercept	2.458		3.227		19.57		52.31	
Area [Control]	-0.302	4.17 <sub>1,63</sub> *	0.60	8.33 <sub>1,63</sub> **	0.87	0.74 <sub>1,61</sub>	-0.75	1.32 <sub>1,61</sub>
Year [2020]	-0.223	2.09 <sub>1,63</sub>	-0.10	0.23 <sub>1,63</sub>	1.00	0.87 <sub>1,61</sub>	1.06	2.41 <sub>1,61</sub>
Female age [Old]	-0.171	1.11 <sub>1,57</sub>	0.065	0.08 <sub>1,57</sub>	0.87	0.59 <sub>1,57</sub>	0.80	1.26 <sub>1,57</sub>
Ambient temperature (°C)	0.039	2.35 <sub>1,61</sub>	0.085	5.48 <sub>1,63</sub> *	-0.36	4.17 <sub>1,61</sub>	-0.49	19.0 <sub>1,61</sub> ***
Measurement time (hour)	-0.078	3.05 <sub>1,62</sub>	-0.032	0.26 <sub>1,61</sub>	0.66	4.66 <sub>1,61</sub> *	0.48	5.96 <sub>1,61</sub> *
Incubation phase (days)	0.096	11.5 <sub>1,63</sub> **	-0.061	2.33 <sub>1,62</sub>	-0.74	12.9 <sub>1,61</sub> ***	-0.35	7.15 <sub>1,61</sub> *

<sup>1</sup> Model reduction is based on AIC values. Terms left in the final model are bolded. \* =  $p<0.05$ , \*\* =  $p<0.01$ , \*\*\* =  $p<0.001$ .

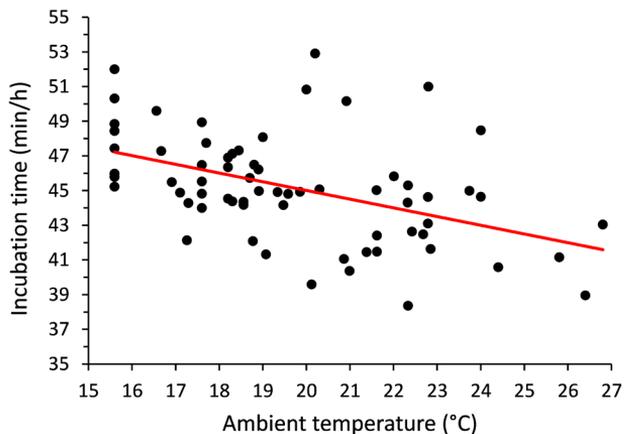


Fig. 5. The association between incubation intensity (incubated minutes per hour) and ambient air temperature (measured by a logger on the outer back wall of the nest box). Linear regression line. N=67 nests.

data. This is because humidity gave a stronger and faster signal as a response to female's incubation behavior, whereas all changes of bouts (most likely those of short duration) could not be detected from the temperature curve. Even though humidity-based and temperature-based values were positively correlated, the video-observations suggest that the humidity-based values reflect better the female movements. However, lower bout frequency and higher bout duration in the temperature-based data have opposite effects on total incubation time, and, at the end, the two methods still gave very similar total incubation intensities. A reason to the higher sensitivity of humidity measure is likely the higher relative variation in the humidity data. In practice, higher variation means stronger peaks in the humidity graph, from which the inflection points will be more easily detected by the NestIQ software.

The justification to measure temperature variation is straightforward, but it is less clear what is the exact mechanism producing behavior-related variation in relative humidity inside the nest. When an incubating female settles in the nest cup, her warm body and possibly breathing are likely to cause an increase in the relative humidity, which is detected faster by the data logger than the temperature, probably because humidity can move fast with air through convection and diffusion whereas heat can move more slowly by conduction through nest materials and the logger capsule. Furthermore, if humidity is evaporated from warming nest materials, this can slow down the increase of temperature because of counteractive evaporative cooling effect. However, further studies are needed to find out whether most of the humidity derives from nest materials (Biddle *et al.* 2019), eggs (Ar & Rahn 1980) or female itself (Dawson 1982). These alternatives could best be tested with experimental studies (*e.g.* by placing an artificial heat source inside an artificial bird nest).

As far as we know, our study is the first to measure humidity variation to record incubation rhythm, and our results suggest that measuring humidity within the nest is a promising technique to test and develop further. A major disadvantage of this technique is perhaps the higher price of humidity loggers, and we do not know if it would work well with other bird nest types (*e.g.* open

nests or ground nests) or nest materials. At least in the case of cavity-breeding *F. hypoleuca*, the humidity-based measurements match better with true incubation behavior, which would enable more precise time-budgets for studying female behavior. Humidity-based measurements will also likely be less sensitive to logger position inside the nest material than temperature measurements. We recommend further testing of the humidity method across different species, habitats, and nest types. However, it may be more crucial to avoid conducting tests in rainy conditions with open nest types.

#### 4.2. Pollution effects based on temperature data

We found that birds in the polluted area took slightly more (11%) but shorter (11%) off-bouts. Because these variables have an opposite effect on incubation intensity, there was no difference in total incubation intensity between polluted and control areas, *F. hypoleuca* females incubating their eggs *ca.* 75% of daytime in both environments. This estimate matches well with what has been found for this species earlier (Lundberg & Alatalo 1992). However, we did not measure possible differences in the birds' daily activity vs. roosting rhythm, which could also affect the total incubation times (see Hope *et al.* 2022). In any case, our results indicate that incubating females in the polluted area do not allocate more time for gathering their energy reserves than the birds in the control area. This could mean that, during the incubation period, *F. hypoleuca* females have similar opportunities to gain enough food and meet their energetic demands in both areas either by their own foraging or supplemental feeding by a male. This species also performs well with rather variable diets (Eeva *et al.* 2005). A recent study from the same area found that the body mass of incubating *F. hypoleuca* females was even slightly higher in the polluted area (Rainio *et al.* 2017). This suggests relatively good food availability in the polluted area, although birds can also maintain higher fat reserves when the availability of food is unpredictable (*e.g.* Lima 1986, Ekman & Hake 1990). Shorter off-bouts might then be explained by smaller energetic constraints in the polluted area.

Consistent with our results, shorter off-bouts were also observed in urban great tits, *Parus major*, which spent more time incubating than those in more remote forest habitats, possibly due to differences in food availability or male investment in female feeding (Amininasab et al. 2017, Hope et al. 2022). We did not record males' food provisioning efforts during the incubation period, but earlier studies in our study area indicated that males at least fed their nestlings with the same frequency and similar food loads in polluted and control areas (Eeva et al. 2005, Mari et al. 2024). Urban blackbirds (*Turdus merula*) and house wrens (*Troglodytes aedon*) again showed more, albeit shorter incubation periods than rural birds (Heppner & Ouyang 2021, Ma et al. 2023). All these studies suggest that urban birds incubate with different rhythm and often with higher bout frequency than rural birds. One possible explanation for this could be an increased anthropogenic disturbance in urban environments (Price 2008). However, birds can also adapt to human disturbance. For instance, urban *P. major* females exhibit bolder behavior during incubation, often remaining on their nests despite disturbances, in contrast to those in non-urban forests (Vincze et al. 2016, 2021).

We also found that *F. hypoleuca* females increased their bout frequency and decreased their incubation intensity over the course of their approximately two-week incubation period. In accordance with this, also black-capped chickadees (*Poecile atricapillus*) showed increased bout frequencies with advancing phase of incubation, likely to keep the egg temperatures more stable for the large embryos (Cooper & Voss 2013). Instead, contrary to chickadees, *F. hypoleuca* females decreased incubation intensity, which might be explained by warming weather during the *F. hypoleuca* incubation period, allowing less intensive incubation with advancing spring and higher temperatures (Lundberg & Alatalo 1992, Amininasab et al. 2017).

In general, and especially after the decreased emissions of pollutants in recent decades, the breeding success of *F. hypoleuca* is currently relatively good in the polluted area of Harjavalta, and important breeding parameters like clutch size, hatching success, and fledgling numbers approach the values of the control area (Eeva

& Lehikoinen 2015, Espín et al. 2016). Still, breeding success remains lower in the polluted area, and especially when there are other simultaneous stress factors during the breeding, such as harsh weather conditions (Eeva et al. 2020). Cold weather during the laying and incubation periods reduces hatchability of eggs in *F. hypoleuca*, and more intensive incubation behavior is likely to be adaptive at lower temperatures (Eeva & Lehikoinen 2010, Eeva et al. 2020). The weather during the incubation periods of *F. hypoleuca* was relatively favorable in the breeding seasons 2020 and 2022, and we found no major pollution-related differences in the incubation behavior. It is, however, possible that such differences could arise under more extreme weather conditions.

#### **Kirjosiepon haudontakäyttötymisen vertailu saastuneen ja puhtaamman ympäristön välillä pesään sijoitettavien lämpötila-kosteusloggereiden avulla**

Ympäristön saastumisen aiheuttamia käyttötymismuutoksia on tutkittu melko vähän, vaikka muutokset eläinten käyttötymisessä saattavat olla varhainen merkki ympäristön heikentymisestä ja niillä voi olla merkittäviä seurauksia eläinten lisääntymiseen. Esimerkiksi huonontunut ravinnon saatavuus saastuneessa ympäristössä saattaa haitata lintujen haudontaa. Monilla lajeilla emolinnut vuorottelevat tyypillisesti pesässä hautomisen ja ravinnonhakumatkojen välillä. Tämän kaksivuotisen (2020 ja 2022) tutkimuksen tavoitteena oli selvittää pienten, linnunpesään sijoitettavien lämpötilaloggereiden avulla, onko hyönteisiä syövä kirjosiepon (*Ficedula hypoleuca*) haudontarytmi erilainen metallien saastuttamalla alueella kuparisulaton ympäristössä verrattuna puhtaampiin vertailualueisiin. Lisäksi testasimme kahden erilaisen mittarin – lämpötilan ja ilmankosteuden – toimivuutta haudontarytmin indikaattoreina. Havaitimme, että vaikka lämpötilaan ja kosteuteen perustuvat haudontarytmiä kuvaavat tunnusluvut korreloivat, kosteuteen perustuvat arvot vastasivat paremmin samanaikaisella videotallennuksella dokumentoitua todellista haudontakäyttötymistä. Tämä johtui

siitä, että loggereiden mittaama kosteuskäyrä reagoi lämpötilaa nopeammin ja voimakkaammin lintujen haudontakäyttäytymiseen. Saastuneen alueen linnut pitivät hieman useampia (11%) mutta lyhyempiä (11%) haudontataukoja, mikä saattoi johtua pienemmästä energiatarpeesta tai paremmasta ravinnon saatavuudesta saastuneella alueella. Haudonnan kokonaisintensiteetissä ei kuitenkaan ollut eroa saastuneen ja kontrollialueen välillä, ja siepponaaraat käyttivät munien hautomiseen 75% ajastaan molemmissa ympäristöissä. Hautovat naaraat eivät siis käyttäneet saastuneella alueella enemmän aikaa energiavarojensa täydentämiseen, eikä munien kuoriutuvuudessakaan ollut eroa suhteessa vertailualueeseen. Ilmankosteutta ei ole tätä tutkimusta aiemmin käytetty lintujen haudontarytmin mittaamiseen ja tulokset osoittavat, että menetelmä toimii hyvin. Lisätutkimuksia tarvitaan esimerkiksi sen testaamiseksi, toimisiko tämä menetelmä erityyppisissä linnunpesissä. Ympäristönsuojelun näkökulmasta tulokset tuovat arvokkaan lisän suhteellisen niukkaan tietoon käyttäytymisen muutoksista saastuneessa ympäristössä.

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