

# Developmental Exposure to Aroclor 1254 Alters Migratory Behavior in Juvenile European Starlings (*Sturnus vulgaris*)

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## Supporting Information

**ABSTRACT:** Birds exposed to endocrine disrupting chemicals during development could be susceptible to neurological and other physiological changes affecting migratory behaviors. We investigated the effects of ecologically relevant levels of Aroclor 1254, a polychlorinated biphenyl (PCB) mixture, on moult, fattening, migratory activity, and orientation in juvenile European starlings (*Sturnus vulgaris*). Birds were orally administered 0 (control), 0.35 (low), 0.70 (intermediate), or 1.05 (high)  $\mu\text{g}$  Aroclor 1254/g-body weight by gavage from 1 through 18 days posthatch and later exposed in captivity to a photoperiod shift simulating an autumn migration. Migratory activity and orientation were examined using Emlen funnel trials. Across treatments, we found significant increases in mass, fat, and moulting and decreasing plasma thyroid hormones over time. We observed a significant increase in activity as photoperiod was shifted from 13L:11D (light:dark) to 12L:12D, demonstrating that migratory condition was induced in captivity. At 12L:12D, control birds oriented to  $155.95^\circ$  (South-Southeast), while high-dosed birds did not. High-dosed birds showed a delayed orientation to  $197.48^\circ$  (South-Southwest) under 10L:14D, concomitant with apparent delays in moult. These findings demonstrate how subtle contaminant-induced alterations during development could lead to longer-scale effects, including changes in migratory activity and orientation, which could potentially result in deleterious effects on fitness and survival.



## INTRODUCTION

Worldwide, many migratory bird species are exhibiting sustained and expansive population declines, prompting research into the likely causal factors influencing mortality during migration. The migratory period is often thought to carry higher mortality risk, which can arise from conditions experienced during flight or at stopover locations.<sup>1</sup> Important avian life history events such as moult and migration are sensitive to seasonal environmental cues with a cascade of internal physiological changes that are under direct hormonal and neurological control. Physiological and behavioral changes associated with migration, such as hyperphagia, premigratory fattening, *Zugunruhe* (migratory restlessness), moult, and energy usage, are regulated by different aspects of the endocrine system.<sup>2</sup> Autumnal migration is thought to be regulated by a number of hormones, such as thyroid hormones, prolactin, and glucocorticoids, while gonadal steroids, such as testosterone, are thought to be of primary importance for vernal (spring) migration.<sup>3,4</sup> Accurate and timely departure, orientation, and navigational abilities are considered key components of successful migration.<sup>5</sup> However, these are potentially vulnerable to subtle impairment of the morphological, physiological, and behavioral traits expressed before and during migration.<sup>6</sup>

Chemical pollutants produced by anthropogenic activity are widespread in the environment and are commonly found in the tissues of wildlife and humans.<sup>7,8</sup> Endocrine disrupting chemicals (EDCs) are a diverse group of compounds that are biologically active and capable of interfering with the action of endogenous hormones and have the potential to disrupt homeostatic systems of organisms at multiple life stages including migration.<sup>9,10</sup> Much of the research examining the range of EDC effects on organisms has focused on mechanistic toxicity pathways and consequent molecular, biochemical, and cellular responses.<sup>11</sup> Studies assessing higher order effects have primarily focused on short-term reproductive end points, despite the potential for latent disruption affecting other important life stages relevant to fitness of avian wildlife populations.<sup>7,12</sup>

Polychlorinated biphenyls (PCBs) are ubiquitous synthetic organic contaminants<sup>8</sup> that were previously manufactured and marketed as commercial Aroclor mixtures.<sup>13</sup> PCBs are pervasive environmental contaminants<sup>14,15</sup> due to their persistence and lipophilic properties that facilitate bioaccumulation and biomagnification through food chains.<sup>8</sup> Well known for their

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developmental neurotoxicity,<sup>16</sup> several PCBs and their toxic hydroxylated metabolites (OH-PCBs) are structurally similar to thyroid hormones leading to wide ranging effects on processes common between humans and experimental animals, such as impairments in growth, learning, cognitive abilities, memory, and behavior. Their ubiquitous occurrence and reported high concentrations in a wide range of avian species<sup>16</sup> may contribute to the disruption of thyroid-dependent developmental processes in avian wildlife.

In birds, normal levels of thyroid hormones are essential for controlling basal metabolic rate, pre- and posthatch differentiation of organ systems, growth, initiation of moult and plumage growth, lipogenesis, and secondary sex characteristics.<sup>17</sup> They are also important for nervous system and brain development.<sup>18,19</sup> Exposure of experimental animals and wildlife to PCBs has resulted in a wide range of effects on thyroid function,<sup>20,21</sup> with consequences for thyroid gland morphology and function, thyroid hormone synthesis, secretion, metabolism, transport, binding, and excretion.<sup>18,22</sup> Results of these studies have led to a rapid rise in wildlife studies reporting thyroid dysfunction as an indicator of environmental contamination.<sup>20</sup> The effects of PCBs and other dioxin-like compounds on brain development (e.g., asymmetry) in birds have been characterized,<sup>23</sup> which could have negative consequences for migratory ability later in life. PCBs can also interfere with hormone signaling at multiple levels of the endocrine system during development, resulting in the potential for effects on sex-specific behavioral and cognitive functions.<sup>24,25</sup>

Our objective was to evaluate how early exposure to a commonly encountered PCB mixture, Aroclor 1254, may cause effects on a key life stage of migration. We used a controlled dosing and captive migration study to evaluate potential latent effects on moult, fattening, activity, and orientation behavior in a migratory songbird model, the European starling (*Sturnus vulgaris*). We hypothesized that subtle alterations in physiological processes from PCB exposure during critical developmental windows may have consequences for successful moult and migration later in life, namely, through interference with the hypothalamic-pituitary-thyroid (HPT) axis.<sup>26</sup> Alterations in the HPT axis can have consequences for impaired thyroid and brain function, which may be expressed in two ways: (1) alterations in physiological and morphological traits in response to changing photoperiod and (2) effects on migratory activity and orientation behavior during a simulated autumn migratory event.

## METHODS

**Study Animals and Husbandry.** In 2012, 34 nestboxes (20.3 cm width, 15.2 cm depth, 70 cm high, entrance hole size of 4.5 cm diameter) were established throughout the University of Saskatchewan's Goodale Research Farm (Saskatoon, Saskatchewan, Canada; 52°3'23.13", -106°30'47.90"). Breeding European starlings (*S. vulgaris*) readily adopt nestboxes and are diurnal migrants, with a well-studied breeding and migratory ecology.<sup>15,27</sup> These characteristics, combined with their intelligence and robustness in captivity, make them highly suitable for examining changes in migratory behavior resulting from exposure to PCBs.<sup>28</sup>

Nestboxes were monitored every 2–3 days for nesting activity and date of clutch initiation (appearance of first egg). The clutch was determined to be complete when a female starling was observed incubating the eggs or if the eggs were warm. At the end of the 12 day incubation period, nests were visited daily to determine precise hatching dates (day 0) and to initiate dosing

(day 1). Individual chicks within each nest were identified by clipping different downy feather tract patterns and were banded on day 7. On day 18, nestling starlings were brought into captivity and caged as a nest group at the Animal Care Unit at the Western College of Veterinary Medicine, University of Saskatchewan. Nestlings were fed an organic wetted mixture of turkey starter crumbles, hard-boiled eggs, pureed carrots, and multivitamin powder (Hagen Prime) 6–8 times a day to satiation until they were able to independently feed on dry turkey starter (approximate age 30–35 d). Color-banded birds were then group housed in a free flight colony room (5 × 3 × 3 m) for 2 months before being randomly assigned to cages with 2–3 birds per cage. Starlings were maintained on *ad libitum* water and turkey starter crumbles supplemented with fresh fruit and mealworms under a summer photoperiod of 15L:09D (light:dark) (lights on 06:00 h) to mimic natural day length until initiation of photoperiod shifts.

**Aroclor 1254 Dosing.** Treatment levels were chosen to mimic an exposure to Aroclor 1254 that would produce subtle effects in the study species, while not producing overt signs of PCB toxicity. Aroclor 1254 was chosen for this study due to an abundance of acute and sublethal toxicity data in avian models<sup>29–31</sup> and because the chromatographic patterns of PCB congeners in wild birds closely resemble this mixture.<sup>32</sup> Tissue PCB residues associated with effects on early development of altricial wild birds can range from 0.20 µg/g in whole eggs of the zebra finch (*Taeniopygia guttata*) to 7.7 µg/g (ppm) wet weight in whole body homogenates of nestling eastern bluebird (*Sialia sialis*),<sup>33</sup> levels of which are comparable to those produced by the target dosing concentrations of Aroclor 1254 in the present study.

Analytical grade Aroclor 1254 (Supelco Analytical, Bellefonte, PA) was dissolved in food-grade organic sunflower oil (Compliments brand, Sobey's Canada) to produce a sunflower oil only control and three dose solutions (50, 100, and 150 ppm). Mean concentrations of Aroclor 1254 for all dosing solution batches were confirmed by chemical analysis (see Supporting Information for more details) as 0 ppm (control) = 0.03 ± 0.00005 µg/mL, 50 ppm (low) = 51.72 ± 0.02 µg/mL, 100 ppm (intermediate) = 85.04 ± 0.02 µg/mL, and 150 ppm (high) = 153.14 ± 0.44 µg/mL. Solutions were made from a stock of 1000 ppm (= 987.47 ± 1.85 µg/mL) (Table S2, Supporting Information).

All chicks in each nest ( $n = 21$  birds/treatment) were randomly assigned to the four treatment groups within each box to account for any nest or heritable effects. Eighty-four birds were orally dosed daily from 1 to 18 days posthatch using a crop gavage needle. Dose solution volumes were adjusted daily according to nestling body mass to maintain the following dosage levels based on nominal targets: 0 (control), 0.35 (low), 0.70 (intermediate), and 1.05 (high) µg Aroclor 1254/g-body weight [bw]/day [d]. On day 19, approximately 24 h postdosing, a subset of starlings ( $n = 5$ ) from each treatment group were euthanized by CO<sub>2</sub> asphyxiation for tissue residue analysis. A total of 55 birds remained for Emlen funnel migration trials. Upon completion of the photoperiod experiments, all remaining starlings were euthanized by CO<sub>2</sub> asphyxiation, and sex was determined by post-mortem examination of the gonads.

**Emlen Funnel Migration Trials.** Plastic flower pots (diameter 38.1 cm, height 16.5 cm) were used to create a modification of the original Emlen funnel<sup>34</sup> to test the birds' migratory orientation. Emlen funnels are circular orientation cages with an ideal slope that ensures a bird is forced to return to

the center after an activity bout, yet large enough so that movement from the center to the edge of the funnel is easily distinguished by observation with overhead cameras. The top opening is covered with a fine-meshed screen, allowing individual birds to see the sky, while a bottom opening is cut into the funnel for easy placement and removal of birds. Funnels were spray-painted white to maximize contrast between the bird and the funnel. A black marking was placed on the edge of each funnel (not visible to the bird), aligned to magnetic north. To track migratory movements, digital cameras (ADS-180, Swann Communications) were securely attached to the end of a 10 ft aluminum pole overhanging the funnels. Each camera recorded the movements from six funnels simultaneously, and five cameras were connected to a single digital video recorder (DVR8-2550, Swann Communications) and a computer monitor.

From September to October, ~4 month old captive starlings were put on a regime of gradually decreasing photoperiod over 6 weeks from 15L:09D, with weekly 1 h time shifts to 09L:15D. The time of light shifting once weekly matched the natural outdoor sunrise over the experimental period but shortened the photoperiod by advancing the daily sunset. The orientation experiments took place outdoors in a large open field with no landmarks visible from the funnels. Birds were allowed to feed in their home cages for 45 min after lights turned on and were then, at random, individually placed into inverted funnels and transported immediately to the experimental site without visual cues. Funnel trials occurred under clear skies and within 4 h of the natural sunrise. Trials lasted for 30 min, with the first 5 min omitted during analysis to allow acclimation to the funnels. Funnel tests occurred over two consecutive days each week during each photoperiod regime, with half of the 55 birds randomly tested on the first day and half tested on the second day (see Table S3, Supporting Information, for breakdown of  $n$ /sex for each trial). A bird was excluded from a trial if it had any sign of injury, typically from damage to flight feathers. A total of six birds were excluded from the first trial (13L:11D) and one bird was excluded from the second trial (12L:12D), with none being excluded on subsequent trials. Activity of the birds in the funnels was recorded using video cameras and analyzed using the procedures described below (see Data Analysis).

**Migratory Condition Measurements.** Body mass ( $\pm 0.1$  g), furcular fat score,<sup>35</sup> and primary moult score<sup>36</sup> were measured twice weekly from the start (15L:09D) to the end of the photoperiod shift (09L:15D). Data collection and recording was conducted by the same researcher to ensure consistency and was blind to experimental treatment. Measurements obtained from each bird took place on days that did not coincide with funnel trials and were averaged for each week. Furcular fat deposits were scored on a scale from 0 to 5 (0 = no fat deposit, 5 = convex bulge of fat in the furcular region). A primary feather moult score for each bird was calculated as the sum of growth scores over nine primary wing feathers. Each primary feather on the right wing was assigned a growth score from 0 to 5 (0 = old feather, 5 = fully grown new feather), for a maximum wing moult score of 45.

**Hormone Analysis.** Blood was collected (maximum 0.3 mL) from each bird on weeks 1, 4, and 6 of the experimental period by venipuncture of the jugular vein using a 26G needle and was transferred to heparinized microcentrifuge tubes. Blood was kept on ice and centrifuged for 5 min (relative centrifugal force ( $g$ ) = 3099) within 3 h of collection to separate plasma. Samples were stored at  $-80$  °C until analysis. Total thyroxine (T4) and total triiodothyronine (T3) concentrations in plasma samples were determined using enzyme-linked immunosorbent assay (ELISA)

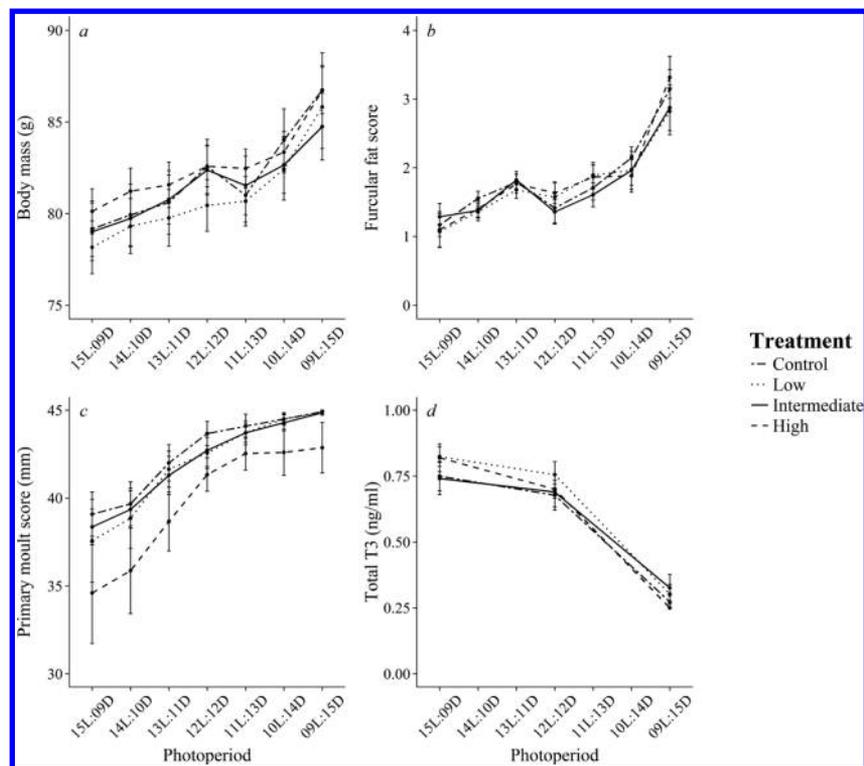
kits (Monobind 225-300 and 125-300, Lake Forest, CA 92630). T4 and T3 were quantified in accordance with protocols provided by the manufacturer. Chicken plasma from an in-house 10 hen plasma pool was included in each plate, and all samples were run in duplicate to measure inter- and intra-assay precision and reproducibility, respectively. The T3 assay had an average inter-assay coefficient of variation (CV) of 5.96% ( $n = 5$  replicates), and the intra-assay CV was 8.81% ( $n = 2$  replicates); however, T4 samples were all below detection ( $<1.28$  ng/mL).

**Data Analysis.** General linear mixed models (GLMM) were used to investigate the effects of Aroclor 1254 treatment on European starling measurements and activity over the course of the photoperiod shifts. Body mass, furcular fat score, right wing chord length, plasma total T3 concentrations, and migratory activity were log- or square root-transformed to meet assumptions of normally distributed residuals and analyzed using Gaussian distributions with the “nlme” package<sup>37</sup> in R version 3.0.0 (R Core Team 2014). Photoperiod, treatment, and sex were fixed effects; nestbox, subject, camera/funnel position, and the response slope over time for each subject were random effects. We used Akaike’s information criterion (AIC) to identify the best transformations and to decide whether to retain slope and/or intercept random effects.<sup>38</sup> As many responses exhibited curvilinear trends, quadratic (Photoperiod<sup>2</sup>) and cubic (Photoperiod<sup>3</sup>) time components were added to the models and retained if they improved model fit, as determined by AIC. Significant interactions were examined using posthoc testing of contrasts in the “phia” package.<sup>39</sup> Data shown in figures represents untransformed means  $\pm$  standard errors of the mean (s.e.m.), and tests were deemed significant at  $\alpha < 0.05$ .

Emlen funnel video files were analyzed with BirdOriTrack software, a custom-designed motion video-tracking program<sup>40</sup> that allows for the simultaneous analysis of 6 birds (funnels) and corrects for camera distortion. This program obtains the vector orientations and distance moved for each bird relative to the center of each funnel by comparing neighboring video frames. Output from the program includes the unimodal and axial mean direction ( $\theta$ ), mean vector length ( $r$ ), the number of counted movements ( $n$ ), and the tracks of the bird for each funnel (total activity). The total number of counted movements ( $n$ ) refers to the number of hops that the bird completes in the funnel during the trial period. The measure of total activity in the funnel takes into account both the presence of a valid hop ( $n$ ) in the funnel and the mean vector length ( $r$ ), which best represents the movements of migratory activity in the funnel. Activity is calculated as the length of each bird’s movement over a set time interval (5 s) relative to the radius of the funnel (radius = 1). Output from BirdOriTrack was used for calculating circular statistics to determine the mean orientation of all birds from each trial and treatment group using Oriana 3.0 software (Kovach Computing Services). A Rao’s spacing U test<sup>41</sup> was first used to compare orientation distributions among treatment groups and trials to determine whether mean orientation differed significantly from a random distribution. A Watson-Williams F-test<sup>42</sup> was used to compare mean orientation vector angles (i.e., degrees) among treatment groups and trials. All values are reported as mean  $\pm$  s.e.m., and tests were deemed significant at  $\alpha < 0.05$ .

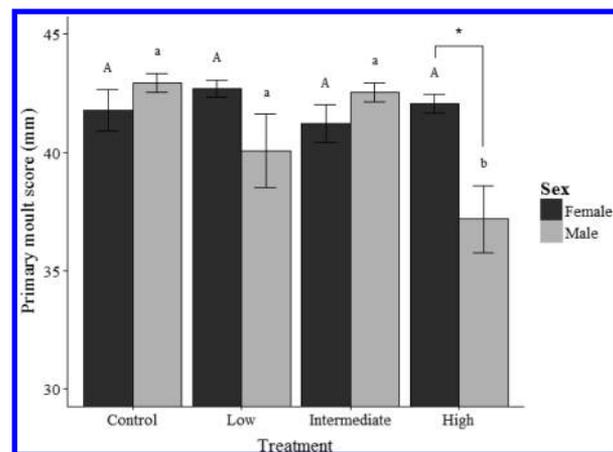
## RESULTS

**Morphological Measurements.** Photoperiod, treatment, and sex were factors included in the most parsimonious models for responses of (Table S4, Supporting Information), fat score



**Figure 1.** (a) Body mass (g), (b) furcular fat score, (c) moult score (mm), and (d) plasma total triiodothyronine (T3) (ng/mL) measured over a 6 week photoperiod shift simulating autumn migration in juvenile European starlings exposed to 0 (control), 0.35 (low), 0.70 (intermediate), or 1.05 (high)  $\mu\text{g}$  Aroclor 1254/g-bw/day. Data represented as mean  $\pm$  s.e.m. There were no significant differences in body mass, fat score, moult score, or T3 levels among treatments (but see Figure 2 for moult score by sex).

(Table S5, Supporting Information), and moult score (Table S6, Supporting Information). Body mass of all birds exhibited a significant curvilinear increase over the photoperiod shift between 15L:09D and 09L:15D ( $\beta \pm \text{S.E.}: 0.042 \pm 0.0091$ ,  $p < 0.001$ ; Figure 1a, Table S4, Supporting Information). Fat scores linearly increased between 15L:09D and 09L:15D ( $\beta \pm \text{S.E.}: 0.23 \pm 0.062$ ,  $p < 0.001$ ; Figure 1b, Table S5, Supporting Information). Sex influenced body mass ( $\beta \pm \text{S.E.}: 0.092 \pm 0.032$ ,  $p = 0.01$ ; Table S4, Supporting Information) but not fat score ( $p > 0.05$ ): males from all treatment groups were significantly heavier than females. Prior Aroclor treatment did not affect fat score, but high dose birds had significantly greater body mass than intermediate and low dose and control birds ( $\beta \pm \text{S.E.}: 0.071 \pm 0.033$ ,  $p = 0.037$ ; Table S4, Supporting Information). Though all birds initiated their moult prior to the start of the photoperiod shift, moult score of all birds showed a curvilinear increase over the photoperiod shift, leveling off after 12L:12D (photoperiod:  $\beta \pm \text{S.E.}: 0.20 \pm 0.051$ ,  $p < 0.001$ ; Figure 1c, Table S6, Supporting Information). Prior Aroclor treatment did not affect total moult score ( $\beta \pm \text{S.E.}: -0.45 \pm 0.36$ ,  $p = 0.22$ ; Figure 1c, Table S6, Supporting Information). However, high-dosed birds showed a slight delay in completion of moult relative to the controls (Figure 1c). This delay in moult completion was attributed to an interaction between treatment and sex ( $\beta \pm \text{S.E.}: -0.28 \pm 0.12$ ,  $p = 0.024$ ; Table S6, Supporting Information). Male high-dosed birds had a significantly lower moult score than male controls ( $\chi^2 = 7.12$ ,  $\text{df} = 1$ ,  $p = 0.01$ ; Figure 2), while females had similar moult scores across treatments. High-dosed males also had lower primary moult scores than high-dosed females ( $\chi^2 = 5.42$ ,  $\text{df} = 1$ ,  $p = 0.02$ ; Figure 2). There were no interactions between treatment and photoperiod/time in any of the tested models (Tables S4–S6, Supporting Information).

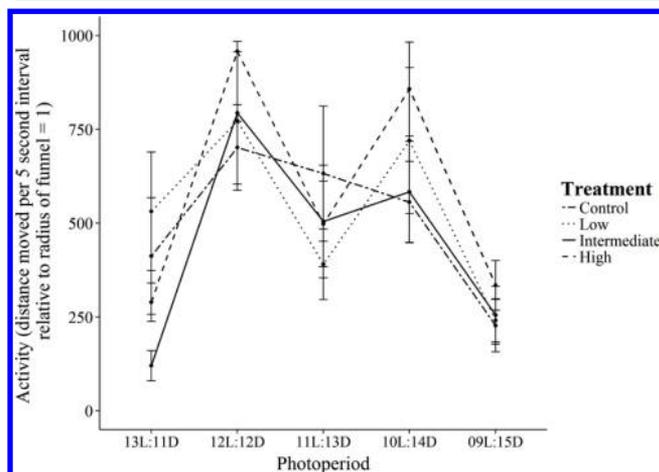


**Figure 2.** Primary moult score (mean  $\pm$  s.e.m) in juvenile European starlings exposed to 0 (control), 0.35 (low), 0.70 (intermediate), or 1.05 (high)  $\mu\text{g}$  Aroclor 1254/g-bw/day measured over a 6-week photoperiod shift simulating autumn migration. Different letters (uppercase = female, lowercase = male) indicate a significant difference ( $p < 0.05$ ) in primary moult score compared to controls of same sex. Significant differences between males and females are indicated as follows: \* $p < 0.05$ .

**Thyroid Hormones.** Plasma total T3 (ng/mL) of all birds showed a significant curvilinear decrease over the duration of the photoperiod shift (Figure 1d, Table S7, Supporting Information). Plasma total T3 was not affected by prior Aroclor treatment or sex (Table S7, Supporting Information). Plasma total T4 values were all below the levels of detection of the assay (1.28 ng/mL).

**Migratory Activity and Orientation.** Migratory activity (total activity) was best predicted by treatment group,

photoperiod, and sex. Activity levels across all birds showed a variable response over time, peaking at 12L:12D and reaching their lowest levels at the end of the experiment under winter daylengths of 09L:15D (Figure 3, Table S8, Supporting



**Figure 3.** Total migratory activity (means  $\pm$  s.e.m.) in juvenile European starlings recorded in five Emlen funnel trials repeated weekly over a 6 week photoperiod shift simulating autumn migration. Starlings were exposed previously as nestlings to 0 (control), 0.35 (low), 0.70 (intermediate), or 1.05 (high)  $\mu$ g Aroclor 1254/g-bw/day.

Information). Aroclor-treated birds appear to exhibit a drop in activity levels at 11L:13D, followed by a second peak in activity at 10L:14D not observed in control birds, but these differences were not significant (no treatment  $\times$  photoperiod interaction; Figure 3, Table S8, Supporting Information). Prior Aroclor treatment did not affect migratory activity, though intermediate dose birds had slightly lower activity levels overall ( $\beta \pm$  S.E.:  $-1.22 \pm 0.66$ ,  $p = 0.07$ ; Table S8, Supporting Information).

Most starlings showed a preferred orientation during one or more trials; however, the direction of orientation differed between photoperiods (Table 1). At 12L:12D, during the peak of migratory activity, birds from the control (Figure 4a), low (Figure 4b), and intermediate (Figure 4c) treatment groups demonstrated a directional preference (Rao's  $U = 177.1$ ,  $175.7$ , and  $183.4$ , respectively;  $n = 12$ ,  $r = 0.17$ ,  $0.54$ , and  $0.38$ , respectively;  $p < 0.05$ ), while birds from the high (Figure 4d) treatment group showed no uniform directional preference (Rao's  $U = 141.0$ ,  $n = 14$ ,  $r = 0.25$ ,  $p > 0.05$ ; Figure 4d). Mean orientation vector among control, low, and intermediate treatments at 12L:12D was similar (Watson-Williams  $F = 1.17$ ,  $n = 53$ ,  $p = 0.33$ ) orienting in a south or southeasterly direction

(Table 1, Figure 4a–c). In contrast, high-dosed birds exhibited a delayed orientation: they oriented to  $197.48^\circ$  (South-Southwest) (Rao's  $U = 171.7$ ,  $n = 15$ ,  $p < 0.05$ ; Figure 4l) only when the photoperiod reached 10L:14D (2 weeks later). All birds in the different dose groups appeared to orient again under 09L:15D (Table 1). However, these movements were in a northerly direction and during the lowest activity levels, suggesting this time period was not appropriate for recording migration and considering the anticipated southward autumnal movements of migratory passerines at these mid-latitudes of the Canadian Prairies.<sup>43</sup>

## DISCUSSION

To the best of our knowledge, this is the first study demonstrating a link between early exposure to EDCs and latent alterations in migratory behavior. Although migratory activity was induced under captive conditions, there was a significant delay in the ability of some PCB-exposed European starlings to correctly orient under simulated autumn migratory conditions. We also found that there was a significant delay in completion of primary moult in male starlings exposed to  $1.05 \mu$ g Aroclor 1254/g-bw (high treatment group) compared to untreated males. This suggests that early PCB exposure may have (1) caused delays in ontogeny related to moult and migratory events and/or (2) impaired neuroendocrine and/or neurocognitive function relating to orientation and navigation. In exploring these potential and nonmutually exclusive hypotheses, we considered the possible role of thyroid hormone homeostasis during migration as a mechanism for controlling these key life cycle events.

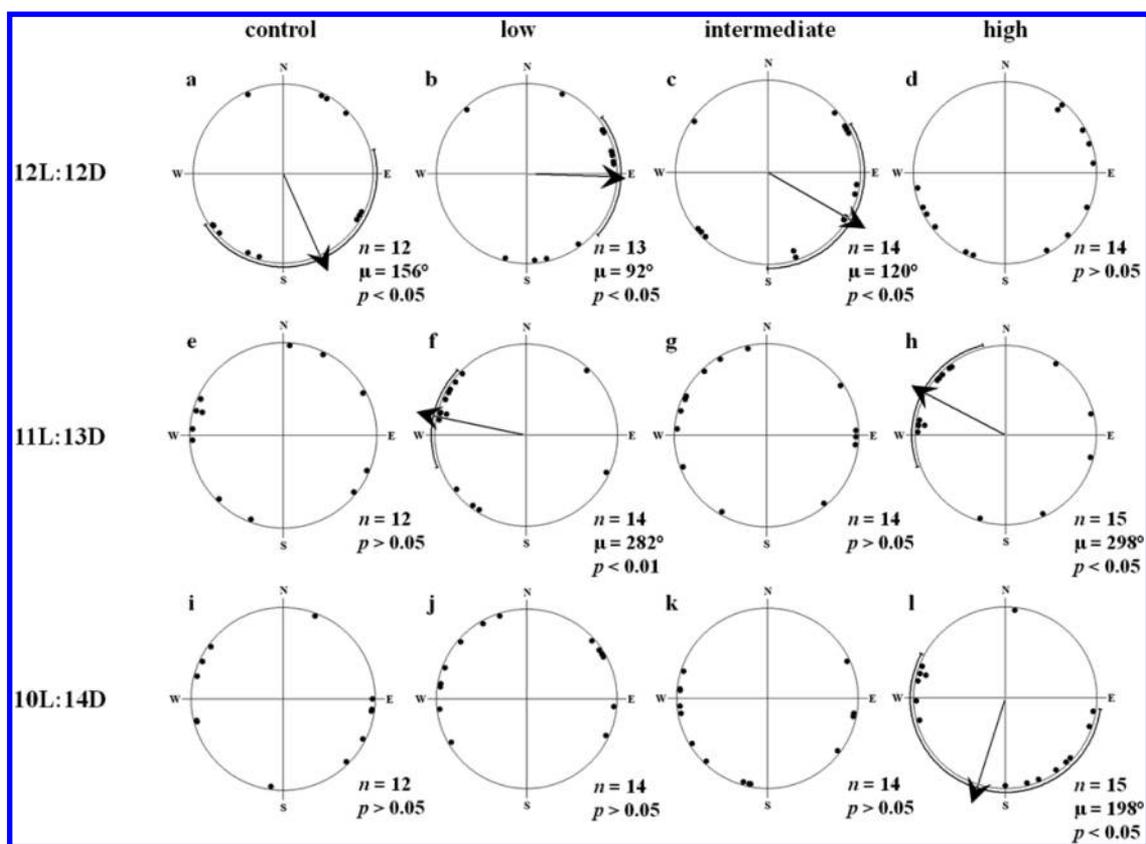
## IMPACTS OF PCB EXPOSURE ON THE ONTOGENY OF A MIGRATION EVENT

Regulation of the timing and manifestation of avian reproduction, moult, and migration in many passerine species is controlled by multiple environmental inputs, primarily seasonal changes in daylength, and intrinsic factors such as endogenous circadian and circannual rhythms within the bird.<sup>44,45</sup> We found that, as photoperiod decreased from 13 h to 12 h of daylight, there was a significant increase in the activity of birds tested in funnels, followed by a continued decrease in activity of controls as the photoperiod reached short winter daylengths, with low, intermediate, and high-dosed birds exhibiting a second peak in activity at 10L:14D. This development of migratory restlessness was accompanied by an increase in body mass and furcular fat deposits, supporting previous findings that this photoperiod (12L:12D) corresponds to the peak of migratory activity in this

**Table 1.** Orientation of Birds under Each Photoperiod and Treatment Level<sup>a</sup>

photoperiod	treatment											
	control			low			intermediate			high		
	<i>n</i>	$\alpha$ (deg)	compass direction	<i>n</i>	$\alpha$ (deg)	compass direction	<i>n</i>	$\alpha$ (deg)	compass direction	<i>n</i>	$\alpha$ (deg)	compass direction
13L:11D	11	n.o.	—	12	n.o.	—	12	n.o.	—	14	307**	NW
12L:12D	12	156*	SSE	13	92*	E	14	120*	ESE	14	n.o.	—
11L:13D	12	n.o.	—	14	282**	WNW	14	n.o.	—	15	298*	WNW
10L:14D	12	n.o.	—	14	n.o.	—	14	n.o.	n.o.	15	198*	SSW
09L:15D	12	334**	NNW	14	327**	NNW	14	333*	NNW	15	337**	NNW

<sup>a</sup>Asterisks (\*) indicate that the bird demonstrated a significant directional preference ( $p =$  significance level according to the Rao's spacing test; \*\* $p < 0.01$ , \* $p < 0.05$ , n.o. = no orientation;  $p > 0.05$ ). Bold indicates the peak in autumn migratory activity observed across treatment groups at the 12L:12D photoperiod cycle.  $n =$  sample size;  $\alpha$  (deg) = mean vector orientation (0–360 deg).



**Figure 4.** Directional orientation of migratory European starlings under 12L:12D, 11L:13D, and 10L:14D experimental photoperiods conducted in modified Emlen funnels. Each dot at the circle periphery represents the mean bearing of one bird, and arrow direction indicates significant migratory vector orientation (mean angle,  $\theta$ ) for each treatment group. Outer curves on each plot indicate the 95% confidence intervals for the group mean orientation (Rao's spacing test;  $p < 0.05$ ), while birds from high treatment group demonstrated a random orientation (Rao's spacing test;  $p > 0.05$ ). Birds from the high treatment group oriented at 11L:13D and 10L:14D; however, orientation at only 10L:14D was deemed correct based on anticipated southerly migratory movements of passerines in our study area.

passerine species at this latitude<sup>45,46</sup> and suggests that birds from all treatments were responding similarly to the change in light regime.

Consistent with the changes in activity, mass, and moult, all treatment groups showed a significant decrease in thyroid hormone concentrations as the photoperiod was shifted from long to short daylengths; however, we found no Aroclor treatment-related effect on circulating levels of T3 throughout the experiment. Both T3 and T4 are important for the entire complement of events associated with migration,<sup>26</sup> directly influencing the initiation of moult and migration, along with the development of premigratory fattening and migratory restlessness.<sup>26,47</sup> While there was an overall acceleration in the completion of moult for all starlings over the duration of the photoperiod shift, male birds from the high treatment group were markedly delayed in this completion and, on average, had not completed a full moult by the completion of the experiment. High-dosed males had a significantly lower moult score when compared to both untreated males and similarly treated females. These results are consistent with a study showing a dose-dependent delay in moult progression of goldfinches (*Carduelis tristis*) during a 4 month daily exposure to environmentally relevant levels of linuron (0–16  $\mu\text{g}/\text{g}\text{-bw}/\text{d}$ ), an herbicide with antiandrogenic and antithyroid activity. Goldfinches from the medium and high treatment groups demonstrated a delay in moult peak, which was mainly attributed to depressed levels of

plasma T4.<sup>48</sup> The key difference between these results and the current study is that a delay in moult progression of high-dosed starlings was exhibited almost 4 months after dosing had been completed, indicating that latent effects resulting from an early exposure to EDCs did persist into adulthood. Unfortunately, in the present study, we were not able to quantify plasma T4 concentrations due to the detection limit of the assay, nor did we measure other candidate hormones involved in moult such as corticosterone or prolactin, which could contribute to observed delays in male moult. Disruption of thyroid hormones and other endocrine regulators of moult and feather regrowth may indicate effects of an exposure to EDCs that persist into adulthood.<sup>48,49</sup> Alterations in hormone function could have direct effects on both initiation and progress of moult in migratory birds, along with carry-over effects on success of migration, breeding, and fitness. Further exploration into the mechanistic underpinnings of hormone disruption during feather development is needed to better understand the significant delays in moult completion observed in the present study.

#### ■ IMPACTS OF EDCs ON AVIAN NEUROENDOCRINE FUNCTION

It has been estimated that over one million tons of PCBs were produced worldwide between 1930 and 1993.<sup>50</sup> Though since banned, PCBs can still be released into the environment through improper waste management, disposal strategies, and leakage<sup>51</sup>

and consequently occur at detectable concentrations in virtually every trophic level of the global ecosystem.<sup>8</sup> There is widespread evidence suggesting that early exposure to PCBs and other similar dioxin-like compounds can result in a range of developmental changes on individuals with consequences for populations of birds,<sup>15,20,52</sup> frequently through alterations in thyroid hormone levels.<sup>20,23</sup> Birds exposed to sublethal concentrations of PCBs in heavily contaminated sites have exhibited alterations in song performance,<sup>53</sup> lower nest quality,<sup>54</sup> impaired reproductive success,<sup>55</sup> and decreased chick survival.<sup>15</sup> Population declines of herring gulls (*Larus argentatus*) from the historically contaminated Great Lakes region is a classic example of population-level effects arising from predominantly PCB contamination.<sup>20</sup>

The treatment ranges chosen for our study represent exposure concentrations at the lower spectrum of contamination which have been shown to produce measurable effects in other avian species.<sup>15,30</sup> Exposure to these low concentrations have also produced measurable effects in other avian studies including in European starlings, through effects on growth, immune function, song output, and behaviors related to breeding and reproduction.<sup>15,56–58</sup> There are a suite of developmental effects that can occur, such as embryonic mortality, teratogenicity, decreased hatching and fledgling success, decreased growth rates, organ maldevelopment, and immunotoxicity,<sup>25,52</sup> all of which can have both immediate and long-lasting effects on chick viability and survival. Some of the more pronounced behavioral alterations arising from exposure to PCB mixtures have included abnormal parenting behavior such as inadequate incubation and nest defense,<sup>59</sup> reduced nest attentiveness,<sup>60</sup> and altered courtship behavior.<sup>61</sup> There is however a lack of information in the literature regarding the effects of exposure to environmental contaminants on migratory behavior in birds. One study exposing adult European robins (*Erithacus rubecula*), a nocturnal migrant, to 11–13 mealworms injected with low levels of Clophen A50 (a PCB) over a period of 2 days, resulted in significantly higher average migratory activity than that of control birds.<sup>62</sup> Migratory restlessness is a critical component of the suite of events preceding a migratory event; however, that study did not examine migratory orientation and only short-term responses following dosing. Further examination of the relationship between PCB exposure and altered long-term developmental processes in birds is crucial for understanding the potential for latent effects on behaviors and life history events that are critical for survival and reproduction.<sup>63</sup>

Increasingly, researchers have recognized the potential for early alterations in thyroid hormone homeostasis and consequent brain morphology resulting in long-term and irreversible changes in brain function, cognition, and behavior;<sup>7</sup> however, there is a general lack of information regarding the impact of EDCs on development of brain regions related to avian migration. Although our study has no direct evidence supporting this, the neurodevelopmental toxicity of PCBs is known to arise from interaction with different processes of brain development, but interference with hormone signaling in the developing brain has been shown to be a predominant pathway through which disruption of thyroid function occurs.<sup>18</sup> Disruption of these essential processes may indicate effects of exposure to PCBs that persist into adulthood, making the brain highly susceptible to embryonic and posthatch EDC exposure.<sup>19</sup> Furthermore, the risk of exposure and sensitivity to PCBs and OH-PCBs, in addition to other structurally similar compounds (e.g., dioxins and polybrominated diphenyl ethers), during critical windows of

embryonic and nestling development is likely greater than that of adults because young individuals undergo large and rapid structural and functional changes. Nestling birds also have a lower body mass, which can contribute to body mass-specific dose effects,<sup>56</sup> making them particularly vulnerable to any long-term toxic effects of chemicals.<sup>25</sup>

In previous displacement experiments during autumn migration events,<sup>64</sup> first-time juvenile starlings revealed different orientation strategies compared to adult migrants. Juvenile starlings possess an innate knowledge of the migratory direction to their wintering grounds. By using a “bearing-and-distance” program (clock and compass orientation) they fly in a fixed direction and distance without specific knowledge about the landmarks of their goal.<sup>65</sup> Juveniles use environmental references such as the earth’s geomagnetic field, the stars, and/or the sun to obtain a direction of reference,<sup>66,67</sup> whereas adults use true goal orientation (homing orientation). The mean migratory orientation (SSE) of control starlings measured under autumn photoperiod conditions (12L:12D) corresponds to that of free-flying local starling migrants.<sup>43</sup> This suggests that examining migratory restlessness and orientation of birds exposed to a 12L:12D photoperiod was the most useful and relevant time period when assessing the effects of an exposure to EDCs on these parameters that directly influence the success of an avian migration event. Equally, because juveniles are using innate orientation, we believe that impairment suggests PCBs are more likely to have affected those brain regions involved in spatial orientation, rather than memory. In contrast, research has demonstrated that migratory orientation could be altered in adult white-throated sparrows (*Zonotrichia albicollis*) upon exposure to acephate, a common organophosphorus neurotoxic pesticide.<sup>68</sup> Control and exposed juveniles moved in a preferred and seasonally correct direction when placed in funnels during a fall migration; however, adults displayed random activity, suggesting that there was some level of interference with neurological function associated with memory of prior migration route and wintering grounds rather than innate compass orientation.

The avian hippocampus plays an important role in spatial processing, learning, and memory,<sup>69,70</sup> which are essential elements in brain regions involved with navigation<sup>71,72</sup> and creation of a spatial map in migratory birds.<sup>73</sup> The hippocampus is a structure sensitive to alterations in hormone levels, including thyroid hormones,<sup>74</sup> and so, further research is needed to elucidate potential alterations in the hippocampi of birds that are impaired in their orientation or navigational abilities. Hippocampal lesions in homing pigeons have resulted in an impaired ability to navigate toward unfamiliar locations, suggesting that any hormone-induced structural changes in the hippocampus could result in the inability of a juvenile passerine to utilize an internal “sun or magnetic compass” during development of a cognitive migration map.<sup>75</sup>

## ■ RELEVANCE TO WILD BIRD POPULATIONS

In the current study, juvenile starlings treated with 1.05  $\mu\text{g}$  Aroclor 1254/g-bw (high dose) failed to orient at 12L:12D, which corresponded to the peak of migratory activity and correct orientation in control birds. High dosed birds displayed a preferred orientation in four out of the five trials (Table 1); however, they frequently misoriented NNW to WNW, directions that are incorrect based on typical migratory patterns of passerines at this latitude.<sup>43</sup> It was not until 2 weeks after the critical time period for migratory activity and orientation (10L:14D) that birds from the high treatment group exhibited

a second peak in migratory activity and oriented SSW, indicating that their timing and accuracy were impaired. This delay, combined with the notable absence of moult completion in birds from the high treatment group, suggests that these birds would be at a distinct disadvantage in the wild when compared to nonexposed individuals. Alterations in the timing and accuracy of migratory events are likely to have negative implications for individual fitness and could result in significant consequences for wild bird populations. Birds can encounter ecological barriers, extreme weather events, and unsuitable habitats during migration, resulting in burdens on their energy and water budgets,<sup>5</sup> making it critical to correctly orient and navigate to essential stopover sites, wintering, or breeding grounds at the appropriate times. Prolonged migration or delays in departure to wintering/breeding grounds resulting from EDC exposure during early development could result in negative carry-over effects such as delayed reproduction, poorer annual survival, reduced food supplies at stopover sites, higher energy expenditure, and increased chances for moving off-course of an established route.<sup>76</sup> Migratory birds that have sufficient fat and body mass reserves but are significantly delayed in their completion of moult and/or orientation ability could remain at a disadvantage when considering the intense amount of competition for resources at stopover sites and breeding grounds.<sup>1</sup>

Migratory birds must also learn and maintain long-term memories associated with stopover sites, wintering grounds, and breeding sites, implying that birds have evolved advanced and innate cognitive abilities required to successfully orient and navigate as juveniles.<sup>66</sup> Although European starlings serve as highly useful models for studies examining ecological effects of exposure to environmental contaminants,<sup>15</sup> they are considered short-distance diurnal migrants that exhibit migration characteristics such as flocking behavior, intermittent short-hops along the route, and reliance upon multiple cues for successful migration.<sup>5</sup> By comparison to some other long-distance, solitary, nocturnal migrants, the starling's life history characteristics likely contribute to their adaptability and success as a population. However, other migratory songbird species could be more strongly influenced by developmental effects on endocrine processes affecting spatial ability and migration behavior.

Some well-known causes of vagrancy in birds include wind drift, autumn 180° orientation (reverse migration), and spring overshooting.<sup>77,78</sup> While weather patterns have been documented to play a role in occurrences of vagrant birds, the degrees to which weather or other factors influence vagrancy are not well understood.<sup>77</sup> Vagrant birds typically originate from migratory populations and are primarily inexperienced juveniles on their first autumn migration,<sup>65</sup> prompting questions as to whether unfavorable weather conditions alone are the cause of vagrancy in juveniles. When confronted with displacement along a migration route, misoriented juveniles cannot perform navigational corrections,<sup>79</sup> decreasing the likelihood of their arrival at a location suitable for resting or wintering. Any errors in navigation, combined with the intense selection pressures that birds face during migration, could result in deleterious consequences for subsequent survival and breeding success.<sup>1</sup> Ultimately, even subtle alterations in the physiological and behavioral mechanisms required for a successful migration could have negative consequences for population viability.<sup>6</sup>

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Additional descriptions of chemical analyses, Emlen funnel trial sample sizes, and general linear mixed model (GLMM) statistical model output. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01185.

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### Notes

The authors declare no competing financial interest.

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