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Effects of Parental and Direct Methylmercury Exposure on Flight Activity in Young Homing Pigeons *(Columba livia)*

John K. Moye[1], Cristina R. Perez[1] & Chris A. Pritsos[1,2]

[1] Department of Agriculture, Nutrition, and Veterinary Sciences, University of Nevada, Reno, Reno, Nevada, USA

[2] Nevada Agricultural Experiment Station, University of Nevada, Reno, Reno, Nevada, USA

Correspondence: Chris A. Pritsos, Nevada Agricultural Experiment Station, University of Nevada, Reno, Reno, Nevada, USA. Tel: 1-775-784-1601. E-mail: pritsos@cabnr.unr.edu

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Abstract

Mercury is one of the most common metals found in contaminated ecosystems. It occurs naturally, but high levels found in contaminated areas derive from human use practices. Among the most vulnerable species to exposure are birds that live, nest, or feed in or near these contaminated ecosystems. Because of the known neurological effects of mercury on birds, it is hypothesized that effects upon migratory ability would be evident after exposure to low levels of this metal, and effects may be exacerbated in young birds. Difficulties in following mercury exposed birds once they migrate away from contaminated areas have left investigators with insufficient data to establish exposure levels causing injury of migratory species due to migration disruption. Breeding pigeons were exposed to ~1.0 mg/kg/day methylmercury via the drinking water, and first round offspring were trained to home after fledging, while also continually exposed to methylmercury. The young pigeons were released individually for three flights, and flight times were assessed and compared to control young pigeon flight times from 3.5, 9, 21, 53, 65, and 98 air miles as well as two individual flights at ~50 air miles from multiple directions. Results indicate that methylmercury exposed birds exhibit slower flight times than controls during the initial flight, and generally improve on successive flights at each distance and direction. This may suggest orientation impairment and allude to migration disruption in migratory species.

Keywords: *Columba livia*, homing pigeon, methylmercury, migration, orientation

1. Introduction

1.1 Mercury Contamination and Effects on Birds

Mercury is a globally distributed contaminant introduced in many ecosystems as discharge from human activities such as agriculture, industry and mining. The organic form of mercury, methylmercury, is of the greatest concern due to its ability to bioaccumulate in animal tissue and concentrate in high levels at the top of the food chain (Wolfe et al., 1998). Methylmercury is the most abundant toxic form of mercury found in biological systems. It is a known neurotoxin that has been shown to disrupt endocrine behavior, and impair physiology and reduce reproductive success in wildlife species (Frederick & Jayasena, 2010). Aquatic species high in the food chain have been reported as being the most at risk due to the bioaccumulation effects methylmercury exhibits through trophic levels. Additionally, birds that live, nest, and feed near contaminated sites are among the most at risk to exposure due to their foraging behavior (Friend & Franson, 1999). Reproductive success in birds has been observed to decrease 35-50% with high levels of mercury exposure even in the absence of any visual impairment in the adults (Brasso & Cristol, 2008). The movement of many avian species during their seasonal migration puts them at risk for mercury exposure while they feed and drink at potentially contaminated sites along their migration route. Successful migrations to feeding, breeding, and wintering grounds are fundamental in maintaining many bird populations. Migratory birds rely on their navigation skills to return home from their temporary migratory residence. A bird's ability to navigate home is an interrelated two-step process composed of establishing the geographic position of the release site and determining the homing direction of flight (Wiltschko & Wiltschko, 2001). Due to the neurological effects mercury has on wildlife species, it is suggested that low level mercury exposure to birds would impair their migratory ability that is essential for a successful migration. However, evaluating the impact of contaminants like methylmercury on migratory birds is difficult. In order to obtain the data needed to evaluate the effects of exposure on their migration bout, the migrants must be followed which is difficult to do once the birds leave the contaminated site.

1.2 Avian Navigation and Homing Pigeons

Homing pigeons (*Columba livia)* have been used as a model species to study avian orientation and navigation. It is suggested that the navigational system of wild birds is based on the same principles of the pigeon's ability to home (Wiltschko & Wiltschko, 2001). One portion of the homing pigeon navigational mechanism is composed of the compass map, which allows the birds to determine their position with respect to the home loft. Pigeons have the ability to recognize previously visited sites based on visual cues, and familiar landmarks (Gagliardo et al., 2001). When homing pigeons are released at familiar sites, they are able to recognize characteristic landmarks that enable them to better fly back home. It is suggested that at the beginning of a homing flight, the birds initially rely on the visual and olfactory cues to position themselves in accordance to home and then they rely on a compass bearing to find the direction home (Biro et al., 2004). Thus, in order to determine navigational impairment, it would be necessary to release the birds from multiple distances and in multiple cardinal directions from the loft. Recent research in our lab has used the homing pigeon model to evaluate the effects of other environmental contaminants such as cholinesterase-inhibiting pesticides (Brasel et al., 2007; Moye & Pritsos, 2010) and chemicals found in mining waste (i.e. arsenic and cyanide) (Brasel, 2005; Brasel et al., 2006), which were found to compromise the migratory ability of the birds. In this study, adult pigeons were pre-exposed to methylmercury in their water source for two weeks and allowed to breed. Their offspring were then continuously exposed to methylmercury, and the effects of exposure on their learning and homing ability were evaluated at different flight distances and in different cardinal directions.

2. Methods

All pigeons utilized in the study were housed, trained, and bred at the homing pigeon research facility located at the Agricultural Experiment Station at the University of Nevada, Reno. These studies were conducted under an approved protocol by the university's animal care and use committee.

2.1 Pairing of Breeders

Pigeons were observed for two weeks to assess pairs of birds exhibiting mating behaviors. Band numbers of paired birds were recorded, and pairs were assigned to one of two groups (control or exposed) based on an even distribution of age of birds. The control group was assigned six pairs and the exposed group assigned seven pairs. Control and exposed breeding pairs were then separated into different breeding lofts equipped with nest boxes, nest bowls, and nesting material to commence breeding.

2.2 Dosing and Breeding

Once the treatment groups were segregated into their respective breeding lofts, dosing began. The MeHg exposed group received a concentration of 7.0 mg/L methylmercury hydroxide (~1.0 mg/kg body weight) in deionized water, which served as their drinking water. Five milliliters of methylmercury (II) hydroxide, 1M (Alfa Aesar, Ward Hill, MA) was dissolved in 995 mL of deionized water, which served as a stock solution. Dosing solutions were then prepared by diluting the stock solution with deionized water to the appropriate concentration. The control group received a vehicle drinking water solution (deionized water and buffer). MES buffer (2-(*N*-morpholino)ethanesulfonic acid, 0.1 M, J. T. Baker, Philipsburg, NJ) was added each day to the dosing solutions at a rate of 10 mL/L to maintain pH. After the initiation of dosing, all eggs laid within the following two week period were culled to allow for accumulation of mercury and expected transfer to young. After the two weeks, all eggs laid were recorded and monitored for progress in development.

2.3 Offspring Training and Learning

Offspring from each group began training for the flight studies shortly after fledging, approximately 30-35 days of age. Each treatment group was provided drinking water similar to that of their parents during the exposure period once training was initiated and remained with that water source throughout the training period. Each group was trained separately in order to monitor their progress in training and to assess whether differences occur in their ability to learn to home from various distances and directions. A strict training regimen was conducted in order to monitor subtle behavioral and learning differences between treatment groups. The regimen consisted of daily procedures related to feeding, familiarity with being crated, entering and exiting the loft, and training flights around the loft.

2.4 Variable Distance Experimental Flights

Following training, young birds (YB's) were banded with an electronic chip ring on the left leg to be timed for homing to the loft with a Benzing Atis Top timer (Munich, Germany) and released at increasingly longer distances beginning at 3.5 miles and sequentially increasing to 9, 21, 53, 65, and 98 air miles. The releases occurred along a northerly route from the home loft. The morning of the flight, YB's were loaded into transport crates according to treatment group and transported by motorized vehicle to the release site. Upon arrival, YB's were allowed to rest and assess the release point visually for 20 minutes. After the rest period had elapsed, an individual bird was randomly selected and released. The next bird was selected randomly from the other treatment group and released after 10 minutes had elapsed or when the previous bird was out of eyesight. This process continued until all YB's were released. The groups were released within the same two hour window at each release date to aid in orientation. As birds returned, times were recorded by the electronic clock at the home loft entrance and entered into a spreadsheet for analyses. Findings were analyzed for differences in flight times among treatment groups according to experimental flight. Three flights were conducted at each distance.

2.5 Variable Direction Experimental Flights

Once the variable distance flights were completed, YB's were taken to different cardinal directions for release to assess whether this would have any different or additional impact on their ability to efficiently home. Pre-flight procedures were similar to those during the variable distance experimental flights. Two flights at each release point were conducted. Flights were from 45 miles south and 50 miles east from the home loft.

2.6 Statistical Analyses

All statistics were performed using Prism 5.0 statistical software (Graphpad, San Diego, CA). Individual flights at each distance and direction were analyzed for time of flight differences between treatment groups by t-test and flights among the same treatment group were analyzed by One-Way Analysis of Variance (ANOVA). Combined flight times for treatment groups at each distance and direction were analyzed by t-test. The difference in flight times between treatment groups, according to corresponding flight number (first, second, and third flights) at each distance were also combined and analyzed.

3. Results

3.1 Breeding of Young Birds

Once breeders paired and began reproducing, one pair from each treatment group did not produce any offspring. These breeding pairs were the youngest birds in each of their respective treatment groups. Since birds naturally paired without manipulation and before dosing began, methylmercury exposure did not play a role in these specific pairs' unsuccessful reproduction. The average number of days of exposure of breeders within the methylmercury exposure group before laying the first egg of a clutch used in the study was 26.3. Because eggs were being culled within the two week period immediately following the initiation of dosing, some of the offspring within the exposed group were the product of the second clutch laid after onset of dosing.

3.2 Variable Distance Flights

Average flight times from each distance during the multiple distance study resulted in a general trend of exposed YB's exhibiting slower overall flight times than control YB's, and significantly slower flight times from 3.5 and 53 miles ($p=0.0205$ and $p=0.0284$ respectively, t-test, Figure 1). Individual flights from each of the release points produced varied results. Exposed YB's exhibited longer flight times when compared to controls during the first flight at every distance, however not significantly except for the first release from 65 miles ($p=0.0209$, t-test, Figure 2). Exposed YB's then generally showed improvement in sequential flights when compared to their respective first flights. Sequential flights from 21 and 65 miles were significantly faster than first flights in exposed YB's ($p<0.05$ and $p<0.01$ respectively, One-Way ANOVA, Figure 2). This trend appeared even more apparent when differences in flight times between the two treatment groups were calculated for each flight and all distances pooled (Figure 3). Results show a consecutive drop in difference in flight time minutes, suggesting that exposed YB's became more efficient in homing as they were released multiple times from the same point.

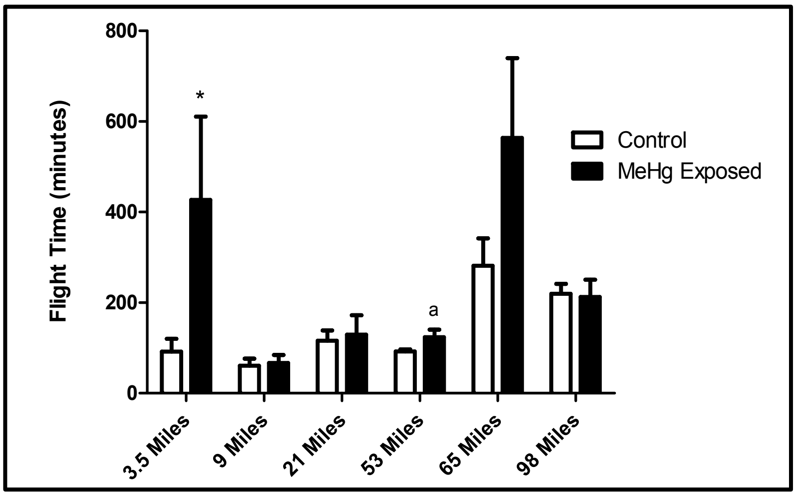

Figure 1. Averaged flight times from control and MeHg exposed pigeons at each distance during the variable distance study

After the training phase, the initial number of birds per treatment group was 7 for controls and 4 for exposed but decreased to 6 for controls and 3 for exposed by the end of the study due to losses. *p=0.021 and [a] p=0.028 vs. respective control, t-test. Error bars = mean±SEM

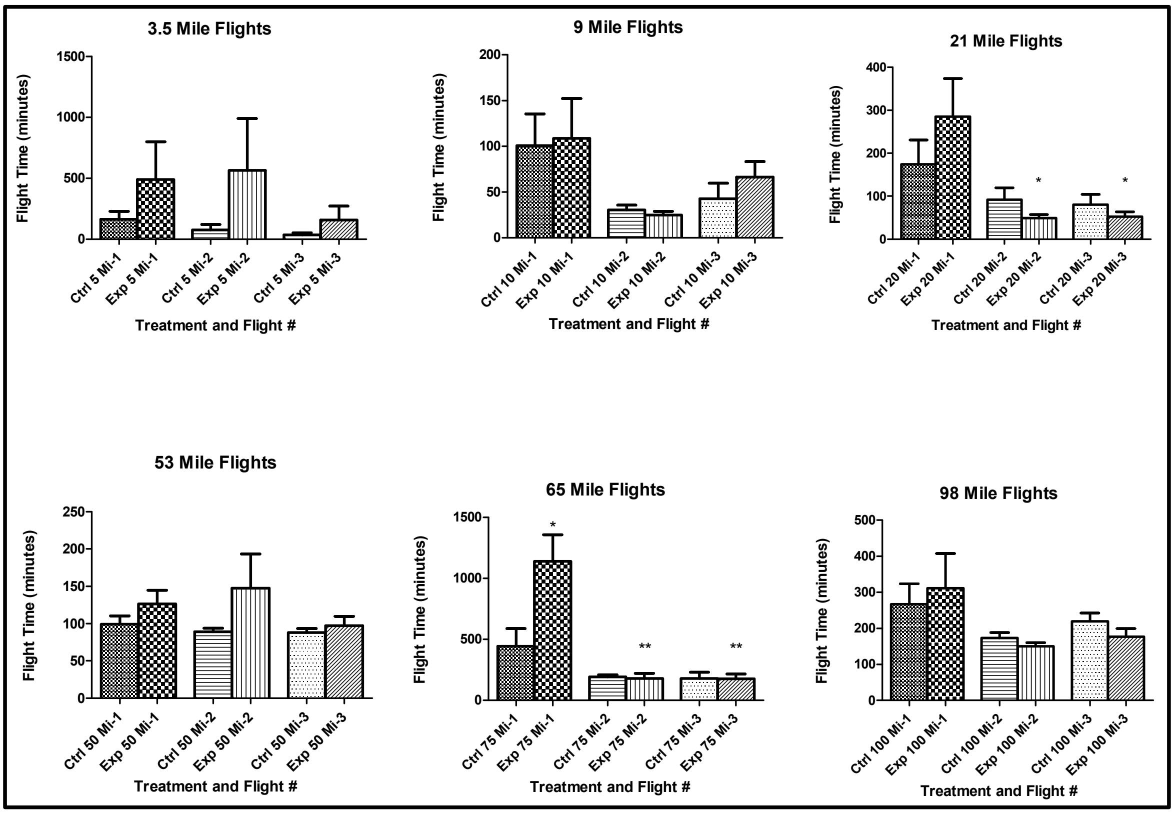

Figure 2. Flight times of three individual flights from control and MeHg exposed pigeons at each distance during the variable distance study

N=7 for controls and N=4 for exposed but decreased to 6 for controls and 3 for exposed by the end of the study due to losses. At 21 miles, exposed ANOVA p=0.016 and *p<0.05 vs. exposed first flight. At 65 miles, exposed ANOVA p=0.004, **p<0.01 vs. exposed first flight and *p=0.021 vs. respective control, t-test. Error bars = mean±SEM

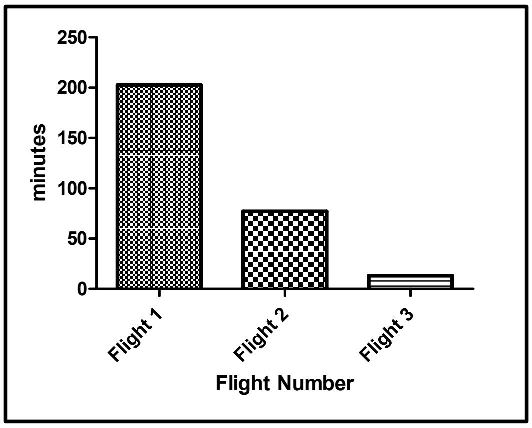

Figure 3. Difference in flight times between treatment groups at each of three flights, all distances combined

3.3 Variable Direction Flights

Flights during the variable direction study resulted in a similar trend as the variable distance study. From both directions, south and east, exposed YB's were considerably slower than controls to home during the first flight from each direction and improved during sequential flights to levels comparable to controls where no statistical difference could be observed (Figure 4). The improvement in flight times from the first to second flight in the exposed treatment group at both directions was statistically significant or approached significance (p=0.047 from south release, p=0.071 from east release, t-test). Low bird numbers during the easterly releases most likely prevented flight time differences from being statistically different. Interestingly, when mean flight times from the first release were compared, exposed YB's were more than two-fold slower from the east than from the south (2298 minutes vs. 1107 minutes) when the distance from the home loft was only a 5 mile difference. This may be due to differences in directional planes. Young birds had no prior experience when released from the east but had flown along the same directional plane when released from the south as they had during the variable distance flights conducted to the north of the home loft.

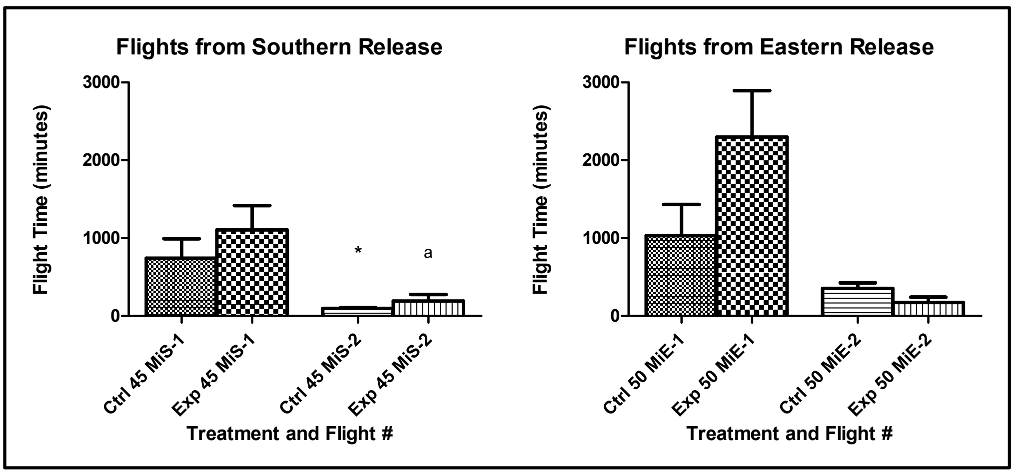

Figure 4. Flight times of two individual flights from control and MeHg exposed pigeons at each direction during the variable direction study.

N=5 for controls and N=3 for exposed for the flights from the southern release point but decreased to 2 birds per treatment group following the first release from the eastern release site. At the southern release, *p=0.034 vs. control first flight and [a] p=0.047 vs. exposed first flight. Error bars = mean±SEM

4. Discussion

Mercury is a toxic metal that accumulates in animal tissues and as it moves up food chains, has the ability to biomagnify. Methylmercury is highly toxic and is the form that is most bioavailable; concentrating at the top of food webs potentially causing severe neurological damage (Wolfe et al. 1998). Although physiological impairment has been the most studied area of methylmercury toxicity in birds, behavioral anomalies may be more indicative of deleterious effects leading to declines in populations. Exposure to methylmercury has been shown to cause subtle behavioral changes in great egrets (*Ardea albus*) (Bouton et al. 1999), common loons (*Gavia immer*) (Nocera and Taylor 1998), and three species of songbirds (Hallinger et al. 2010). In the current study, young homing pigeons displayed signs of flight behavior impairment when exposed prenatally and continually after hatching. Exposed YB's exhibited a general trend of slower flight times than controls during the initial flight, and improvement on successive flights at each distance and direction.

The training period of the study resulted in a number of visually observable differences between the two treatment groups and learning deficiencies within the methylmercury exposed group. Although observations were not quantified, they may suggest preliminary effects that would surface later during the experimental flights. A number of exposed chicks were observed being fed by their parents even after fledging. Pigeons normally wean right around the time they fledge, between 30 and 35 days. These same chicks were exhibiting begging behavior well after fledging. The behavior of delayed weaning may allude to developmental toxicity. Exposed YB's also showed delays in learning during the trap and perch flight aspect of flight training. Birds from both treatment groups were given time to become comfortable with exiting and entering the loft through traps located at the entrance of the loft, just above the flight landing perch. On average, exposed YB's took a week longer to become accustomed with re-entering the loft after exiting. Once the YB's were comfortable with this, they were allowed to leave the loft on their own will and begin developing a visual map of the home loft area while in flight. Again, exposed YB's were generally a week later in attempting flight than control YB's. Exposed YB's were more reluctant to leave the loft and more inclined to immediately re-enter through the traps. Additionally, once crated flights began, exposed YB's were hesitant to leave the crate to initiate a flight. After being transported to a release sight, crates were opened and birds were allowed to exit. Exposed YB's during initial training flights did not willingly leave the crate and had to be physically removed. Learning deficiencies and lack of motivation to fly during training may be symptoms of methylmercury toxicity and if so, would infer that behavioral anomalies became apparent even before the first attempt at flight in the exposed group. Pre- and post-fledging developmental behavior toxicity from methylmercury exposure will require further investigation.

Results of flights revealed an interesting phenomenon and shed some light on the orientation and navigation mechanisms which may be affected by exposure to neurotoxic environmental contaminants, specifically methylmercury. As was the case in the training phase leading up to experimental flights, exposed YB's exhibited a delay in learning demonstrated by an overall trend of less efficient homing during initial flights from each release point, and showed an improvement during sequential flights. This was illustrated by slower flight times in exposed YB's followed by shorter, faster flights. Because avian navigation is comprised of a two-stage process, methylmercury has the potential to affect either or both of these components. At the start of the homeward journey, birds use magnetic, olfactory, or visual cues to position themselves with respect to home, then they recall a previously memorized compass bearing leading them to their final "home" destination (Biro et al. 2004). The pattern of slower flight times in exposed YB's with respect to controls followed by faster, in some cases significantly faster, flight times would lend itself to one of two theories as to the navigation mechanisms which may be hindered. First, because exposed YB's are able to improve on sequential flights, it seems unlikely that recognition of visual cues is being inhibited. Therefore, either magnetic or olfactory cues, or both, are not able to be picked up on as efficiently as in controls. Magnetic orientation requires the recognition of geomagnetic fields and the ability to derive these into an inclination 'compass'. Birds calculate directional information from a pole or equatorial sense rather than cardinal direction (Wiltschko and Wiltschko 1996). Neurotoxic exposure may hinder this ability. In the case of olfactory orientation, homing pigeons are able to guage their displacement from the loft by picking up odors in prevailing winds (Waldvogel 1987). Olfactory receptors may be underdeveloped or blocked from continuous exposure to methylmercury starting prenatally. Homing pigeons have been shown to be less efficient in homing when forced into a state of anosmia (Guilford et al. 1998) and when exposed to artificial sources of air (Gagliardo et al. 2011). Secondly, homing pigeons may have difficulty with memorization, or a "forgetfulness", with respect to the learned compass bearing when exposed to methylmercury. Exposed YB's showed delays in initial flight times from release points along the same northerly route during the multiple distance study, suggesting that from one release point to the next, birds were unable to retain the proper bearing for successful homing. Further investigation into this theory will be necessary, employing Geographic Positioning System (GPS) technology. Flight tracks of pigeons could be recorded and analyzed using GPS in order to determine whether or not compass bearings toward the home loft deviate from controls. A stronger case may be made for the first theory given the results of the variable direction study when initial flight times in the exposed group from the eastern flight were increased more than two-fold from initial flight times from the south. This suggests that when birds are released from a different directional plane, they are unable to decipher inclination and displacement cues as readily.

Migration is a critical part of the sustaining and survival of numerous avian species. Orientation and navigational deficiencies in a first year migrant, making the journey for the first time, may potentially lead to delayed arrival and a reduced likelihood of survival. Any disruption in the migratory cycle, from breeding to wintering grounds and back, may be detrimental enough to cause declines in populations. Pigeon homing used as a surrogate for migration has successfully demonstrated that exposure to methylmercury in young birds can cause delays in homing which may allude to deficits in learning and development of critical skills used in navigation. With the use of this model, mechanisms behind migratory disruption leading to population level effects may therefore be examined more critically.

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