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ASSOCIATIONS BETWEEN REGIONAL DIFFERENCES IN POLYCHLORINATED BIPHENYLS AND DICHLORODIPHENYLDICHLOROETHYLENE IN BLOOD OF NESTLING BALD EAGLES AND REPRODUCTIVE PRODUCTIVITY

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Abstract—The relationship between regional reproduction rates of bald eagles (*Haliaeetus leucocephalus*) and concentrations of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and total polychlorinated biphenyls (PCBs) in blood plasma from nestling bald eagles was assessed. Blood was analyzed from 309 nestlings from 10 subpopulations of eagles across the Great Lakes region. Geometric mean concentrations of *p,p′*-DDE and total PCBs were inversely correlated to the productivity and success rates of nesting bald eagles within nine subpopulations. Nestlings eight weeks of age and older had significantly greater geometric mean concentrations of total PCBs and *p,p′*-DDE than nestlings less than eight weeks of age. The ability to use measurements of *p,p′*-DDE and total PCBs in nestling blood to determine the potential impact of these contaminants on adult nesting on a regional scale was demonstrated.

Keywords—Bald eagle Dichlorodiphenyldichloroethylene Polychlorinated biphenyls Great Lakes Reproduction

INTRODUCTION

Populations of bald eagles (*Haliaeetus leucocephalus*) populations in North America have increased in numbers of breeding pairs since the ban of DDT and other organochlorine compounds in the 1970s. The lessening of egg-shell thinning effects of DDT's metabolite, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), has been a major reason for the current resurgence of bald eagle populations in temperate North America [1–3]. However, the recovery has not been uniform and several regions exist, including the basin of the Laurentian Great Lakes, where populations are not reproducing at a level considered to be healthy [3]. Within the eagles nesting along the shorelines and islands of the Laurentian Great Lakes, *p,p′*-DDE and polychlorinated biphenyls (PCBs) have been linked to poor reproductive success [4–6]. With proposals to alter the status of the eagle under the Federal Endangered Species Act [7] as a result of the increasing numbers of breeding pairs in the contiguous United States, it is important to understand the dynamics of the population recovery and the role of PCBs and similar toxicants, as well as *p,p′*-DDE, as part of this decision.

Bald eagles are sensitive to some types of chlorinated hydrocarbon compounds, especially polychlorinated diaromatic hydrocarbons. For instance, exposure to these contaminants causes teratogenic effects and impairs viable egg formation [8–11]. The bald eagle has been proposed by the International Joint Commission as a biological indicator species of toxic effects of organochlorine compounds on piscivorous wildlife and the effects of bioaccumulation and biomagnification in the Great Lakes [12]. Eagles forage primarily on fish and other vertebrates associated with coastal Great Lakes, riverine, and interior aquatic systems [4,5]. Concentrations of *p,p′*-DDE and PCBs in the plasma of nestlings reflect their exposure to these compounds from the prey species within their breeding area [5,13]. To determine current relationships between concentrations of PCBs and *p,p′*-DDE in bald eagles and reproductive success, we measured concentrations of these compounds as well as several other organochlorine insecticides in plasma of nestling bald eagles and compared concentrations with bald eagle productivity between 1987 and 1992 in 10 subpopulations within the Great Lakes region.

STUDY AREA

The study area contained 10 subpopulations (Fig. 1). Subpopulations from Lake Superior (LS), Lake Michigan (LM), Lake Huron (LH), and Lake Erie (LE) were within 8.0 km of the U.S. and Canadian shorelines of the Great Lakes and along tributaries where anadromous fish were accessible, and are defined as Great Lakes subpopulations. The other six subpopulations, from the lower peninsula (LP) of Michigan, the eastern upper peninsula (EUP) of Michigan east of U.S. Highway 41, the western upper peninsula (WUP) of Michigan west of U.S. Highway 41, the Chippewa National Forest (CNF) of

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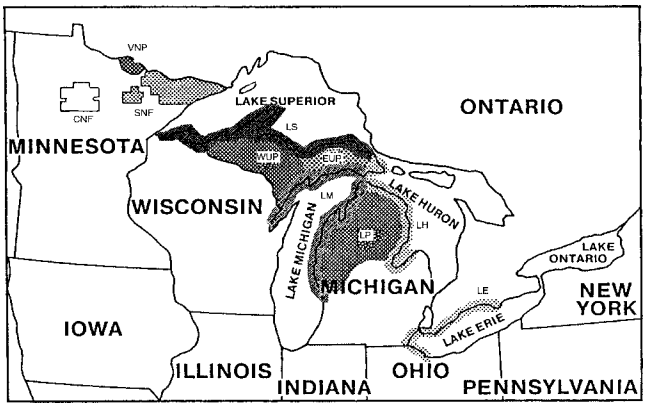

Fig. 1. Ten subpopulations used for comparison of total polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene concentrations in plasma of nestling bald eagles in the Midwest. Locations were within 8.0 km of Lake Superior, Lake Michigan, Lake Huron, and Lake Erie; northern lower peninsula (LP), eastern upper peninsula (EUP), western upper peninsula (WUP) of Michigan; Chippewa National Forest (CNF) and Superior National Forest (SNF), and Voyageurs National Park (VNP), Minnesota, USA.

Minnesota, the Superior National Forest (SNF) of Minnesota, and Voyageurs National Park (VNP) in Minnesota (USA), were at locations greater than 8.0 km from the shorelines of the Great Lakes and not along tributaries where anadromous fish were accessible, and are defined as interior subpopulations.

Subpopulations were geographically distinct areas within the Great Lakes region. Great Lakes subpopulations (LS, LM, LH, and LE) were eagles that preyed upon fish, mammals, and birds that were totally or partially associated with the Great Lakes food web. Potential fish prey from this area previously had been determined to have much greater concentrations of both *p,p′*-DDE and total PCBs than more interior subpopulations [14]. Interior subpopulations were geographically separated (i.e., more than 40 km between the EUP and WUP had no eagle nests, or on a separate peninsula for LP) in Michigan (LP, EUP, and WUP), or were both geographically separated and distinct federal jurisdictional boundaries in Minnesota (CNF, SNF, and VNP). We previously found that incidence of fish, mammalian, and avian prey species collected from nests within these subpopulations are not significantly different among subpopulations [5].

MATERIALS AND METHODS

Blood plasma collection and sex and age determination

Blood was collected from 309 nestling bald eagles between 1987 and 1992. The number of samples analyzed from each subpopulation were: LS, 45; LM, 25; LH, 12; LE, 35; LP, 49; EUP, 16; WUP, 48; SNF, 15; CNF, 43; and VNP, 21. Nestling eagles were sampled during banding activities. A climber would climb up the tree to the nest, then lower one nestling to the ground for blood collection. Sterile techniques were used to collect blood from the brachial vein with heparinized glass syringes fitted with 22- or 24-gauge needles. The syringes previously had been washed with hexane and acetone. Samples of whole blood were transferred to heparinized vacuum tubes, kept on ice in coolers, and centrifuged within 48 h of collection. Blood plasma was decanted and transferred to vacuum tubes and frozen. We determined the age and sex of nestlings by measuring the eighth primary feather and foot pad of nestlings and using these measurements in mathematical growth rate and sexual dimorphism equations [15].

Quantification of chlorinated hydrocarbons

Two different laboratories measured concentrations of total PCBs and organochlorine insecticides with comparable methods [16]. The Environmental Laboratory of the Michigan Department of Public Health (Lansing, MI, USA) analyzed samples collected in 1987 through 1989. The Michigan State University Aquatic Toxicology Laboratory (East Lansing, MI, USA) analyzed samples collected after 1989. Methods used by both labs were nearly identical. Interlab comparisons and validations were accomplished by using spiked bovine serum. Recoveries of organochlorine pesticides and Aroclor® 1254 (Monsanto, St. Louis, MO, USA) from this serum averaged 92 and 87%, respectively [16].

At the Environmental Laboratory of the Michigan Department of Public Health, individual 2- to 4-ml samples of plasma were dissolved in methanol and extracted twice with 5 ml of a 1:1 (v/v) mixture of hexane:ethyl ether by agitating on a rotary mixer for 20 min at 50 to 55 rpm. Extracts were concentrated on a hot water bath to a volume of 0.5 ml. Cleanup was done on a 7-mm Chromaflex column (Kontes Glass, Vineland, NJ, USA) packed with 2.5 g of Florisil (Fisher Scientific, Fairlawn, NJ, USA) by using 10 ml of hexane. Elution of PCBs and chlorinated hydrocarbon pesticides from the Chromaflex column was accomplished with 20 ml of 6% ethyl ether:hexane. Elution of dieldrin from the column was accomplished with 20 ml of 20% ethyl ether:hexane. Separation of PCB from the chlorinated hydrocarbon pesticides was accomplished with a Chromaflex column packed with Silica Gel 60 (Merck, Darmstadt, Germany). The fraction containing hexachlorobenzene and mirex was eluted with 15 ml of hexane. Aroclor 1260, Aroclor 1016, and polybrominated biphenyl were eluted with an additional extraction with 20 ml of hexane. Elution of Aroclor 1016 and chlorinated hydrocarbon pesticides was accomplished with 20 ml of benzene [17]. Concentrations of organochlorine pesticides and PCBs were determined by gas chromatography with confirmation of pooled samples by mass spectrometry [17,18]. Gas chromatography was performed on a Varian 3700 gas chromatograph equipped with small-volume pulsed ^{63}Ni electron capture detector, Varian 8000 Auto Sampler, and Chromatography Data Station-111 microprocessor (Varian, Walnut Creek, CA, USA). A 1.83-m × 0.64-cm × 2-mm-inner diameter glass column packed with 3% SE-30 (General Electric, Cleveland, OH, USA) was used. Nitrogen flow rate was 30 ml/min through the column during operation. Total PCB concentrations were determined on the basis of mean weight percent factors [19]. The following compounds were identified by reference to the relative retention time of *p,p′*-DDE × 100 and were quantified by comparison to authentic standards [17]: 1,1′-(2,2,2-trichloroethylidene)bis[4-chlorobenzene] (*p,p′*-DDT) and its metabolites *p,p′*-dichlorodiphenyldichloroethane [*p,p′*-DDD] and *p,p′*-DDE, hexachlorobenzene, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, dieldrin, polybrominated biphenyls, toxaphene, mirex, alpha-chlordane, and gamma-chlordane.

Analytical methods of the Michigan State University Aquatic Toxicology Laboratory were described previously [16]. Total concentrations of PCBs were determined by congener summing. Individual PCB congener concentrations were determined from response factors and a gravimetric calibration mixture obtained from Columbia National Fisheries Contaminant Laboratory (Columbia, MO, USA). The calibration stan-

Table 1. Productivity, geometric mean, range, and frequency of detectable concentrations of total polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in plasma of 309 nestling bald eagles from 10 subpopulations in the upper Midwest, 1987–1992

			Total PCBs			*p,p′*-DDE		
Area	Productivity	*n*	Geometric mean (μg/kg)	Range	Frequency (%)	Geometric mean (μg/kg)	Range	Frequency (%)
CNF	0.99	43	7	<10–67	23	3	<5–29	19
SNF	1.28	15	5	<10–18	7	3	<5–8	13
VNP	0.80	21	47	<10–1,615	91	20	<5–206	95
LP	1.17	49	31	<10–200	96	10	<5–193	86
EUP	1.04	16	32	<10–146	94	12	<5–24	94
WUP	0.94	48	25	<10–177	88	10	<5–245	79
LS	0.82	45	127	12–640	100	25	<5–306	89
LM	0.63	25	154	14–628	100	35	<5–235	100
LH	0.61	12	105	5–928	100	25	<5–78	92
LE	0.91	35	199	81–1,325	100	22	<5–429	100

[a] CNF = Chippewa National Forest of Minnesota (USA); SNF = Superior National Forest of Minnesota; VNP = Voyageurs National Park; LP = lower peninsula; EUP = eastern upper peninsula; WUP = western upper peninsula; LS = Lake Superior; LM = Lake Michigan; LH = Lake Huron; LE = Lake Erie.

dard consisted of a 1:1:1:1 (v/v/v/v) mixture of Aroclors 1242, 1248, 1254, and 1260. Relative response factors were calculated relative to an internal standard, PCB 30. Several congeners eluted from the gas chromatograph as unresolved peak pairs. In these cases, the combined congener mass was used to calculate the response factor for the peak pair. Total concentrations of PCBs were determined by summing individual masses of the congeners. The following compounds were identified by reference to the relative retention time of *p,p′*-DDE × 100 and were quantified by comparison to authentic standards [17]: *p,p′*-DDT and its metabolites (*p,p′*-DDD and *p,p′*-DDE), hexchlorobenzene, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, dieldrin, polybrominated biphenyls, toxaphene, mirex, alpha-chlordane, and gamma-chlordane.

Reproduction analysis

We calculated productivity (i.e., total number of fledged young per occupied nest) and success rate (percent of nests producing at least one fledged young) for bald eagles for all breeding areas within 10 subpopulations (Fig. 1) from 1977 through 1992 by use of the method of Postupalsky [20]. Subpopulations were analyzed by combining all data for 1987 through 1992 to determine productivity and success rates. Productivity within each subpopulation was determined by dividing the total number of young by the number of occupied breeding areas [20]. Success was determined by dividing the number of nests producing fledged young by the number of occupied breeding areas [20]. Productivities or success rates and the percent of nests producing two or more young were correlated with concentrations of chlorinated hydrocarbons.

Table 2. Geometric mean, standard deviation, and range of total polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in plasma of 118 nestling bald eagles by age in weeks, determined by eighth primary feather measurement [15], 1987–1992. Different letters within columns signify significant differences among ages

		Total PCBs		*p,p′*-DDE	
Age in weeks	*n*	Geometric mean (μg/kg)	Range	Geometric mean (μg/kg)	Range
<5	24	46 C	<10–628	8 C	<5–52
5	24	40 C	<10–160	12 B	<5–41
6	22	41 C	<10–206	11 B	<5–12
7	26	39 C	<10–196	11 B	<5–73
8	16	72 B	<10–158	19 A	<5–43
>9	6	175 A	<10–640	18 A	<5–35

Data analysis

For statistical analyses, all concentrations of chlorinated hydrocarbons were converted to geometric means. Concentrations of *p,p′*-DDE and PCBs were compared statistically among subpopulations or nestling ages by the Kruskal–Wallis nonparametric one-way analysis of variance, or between sexes by the Wilcoxon rank sums test (NPAR1WAY procedure, SAS®/STAT 6.03) [21]. Differences among individual subpopulations or ages were tested by the Kruskal–Wallis multiple range test [22].

Relationships between geometric mean concentrations of PCBs or *p,p′*-DDE in plasma of nesting eagles and productivities or success rates for the nine subpopulations were determined by general linear models for regression analysis (PROC GLM, SAS/STAT 6.03) [21].

RESULTS

Concentrations of PCBs and *p,p′*-DDE varied among subpopulations (Table 1). Geometric mean concentrations of PCBs in plasma of nestlings from Great Lakes breeding areas (LS, LM, LH, and LE) were significantly greater ($p = 0.0001$) than those from VNP, or from other interior subpopulations of Michigan and Minnesota. Geometric mean concentrations of *p,p′*-DDE in plasma of nestlings from Great Lakes (LS, LM, LH, and LE) and VNP breeding areas were significantly greater ($p = 0.0001$) than those from other interior subpopulations of Michigan and Minnesota. Geometric mean concentrations of PCBs and *p,p′*-DDE were significantly greater in plasma of nestlings that were older than eight weeks of age (PCBs, $p = 0.0050$; *p,p′*-DDE, $p = 0.0245$; Table 2) compared to concentrations in plasma of younger nestlings. Geometric mean concentrations of PCBs and *p,p′*-DDE in plasma of nestling eagles were similar between sexes (PCBs, $p = 0.3340$; *p,p′*-DDE, $p = 0.6362$).

All productivity measurements were significantly and inversely correlated with geometric mean concentrations of

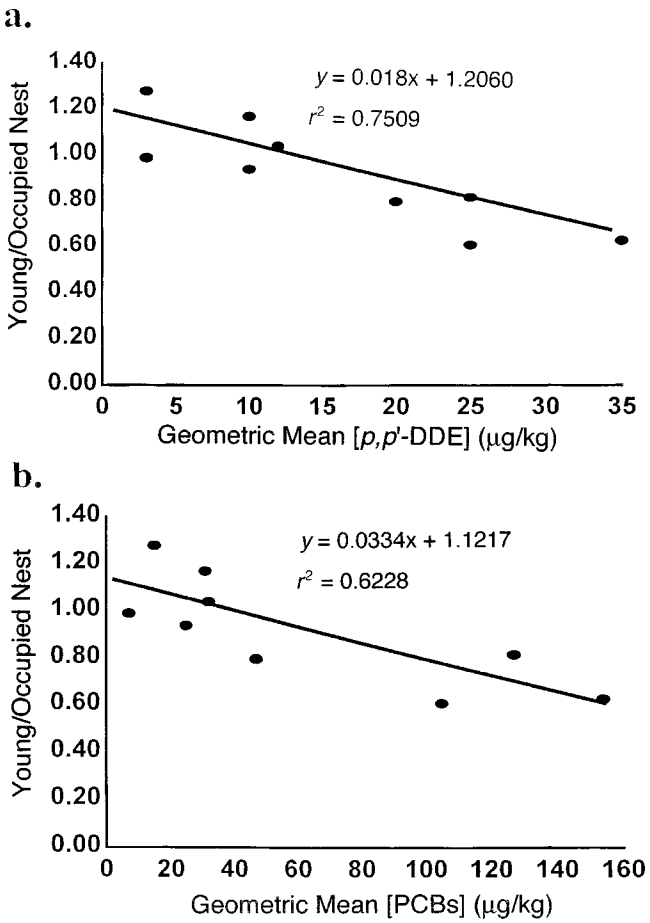

Fig. 2. Relationship between productivity, 1987–1992, and geometric mean concentrations of (**a**) *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), or (**b**) total polychlorinated biphenyls (PCBs) in plasma of nestling bald eagles within nine subpopulations in the upper Midwest. Because of significant differences in results based on age of nestlings, Lake Erie was not included in these analyses.

PCBs and *p,p′*-DDE in plasma of nestling eagles. Productivity within subpopulations was significantly and inversely correlated with geometric mean concentrations of *p,p′*-DDE ($p = 0.0016$, $r^2 = 0.730$; Fig. 2a) and PCBs ($p = 0.0114$, $r^2 = 0.623$). The ability to produce two or three young within subpopulations was significantly and inversely correlated with geometric mean concentrations of *p,p′*-DDE ($p = 0.0030$, $r^2 = 0.688$) and PCBs ($p = 0.0209$, $r^2 = 0.557$). Success rates within subpopulations were significantly and inversely correlated with geometric mean concentrations of *p,p′*-DDE ($p = 0.0023$, $r^2 = 0.707$) and PCBs ($p = 0.0154$, $r^2 = 0.592$).

Statistical analyses for productivity were conducted without the Lake Erie subpopulation because the nestlings sampled were the only nestlings greater than eight weeks of age. These older nestlings had significantly greater concentrations of PCBs and *p,p′*-DDE in blood plasma than concentrations in younger nestlings from all other subpopulations (Table 2). The sampling of these nestlings occurred at 10 weeks of age, an age where growth was complete and the placement of radio transmitters on these nestlings would be less likely to cause problems in flight. Additionally, the samples from these nestlings had a ratio of PCBs: *p,p′*-DDE that was more than two times greater than any other subpopulation, and observations of adult replacement within these breeding areas every five years (M. Shieldcastle, Ohio DNR, Oak Harbor, OH, USA, unpublished data).

DISCUSSION

Production of bald eagles has been demonstrated to be inversely correlated with concentrations of both PCBs and *p,p′*-DDE [8]. Reproduction of bald eagles is considered to be healthy when productivity, measured as young per occupied nest, is 1.0. A productivity of 0.7 is necessary to maintain population stability [23]. Bald eagles that use Great Lakes nests and VNP breeding areas are significantly less productive than eagles that use interior nests in Michigan or Minnesota [5]. However, the relationship between concentrations of PCBs in plasma and productivity is not as strong as for *p,p′*-DDE. This is primarily due to concentrations in plasma samples collected within the LE subpopulation. These samples were influenced by three factors. Firts, the majority of nestling eagles greater than eight weeks of age are within this sample. Second, adult turnover rate within five years of beginning nesting within this subpopulation is documented to be greater than in other subpopulations (P. Hunter, Ontario Ministry of Natural Resources, Aymler, ON, Canada, personal communication). Third, the LE population of nestling eagles is exposed to greater concentrations of PCBs than of *p,p′*-DDE.

The lower productivity of bald eagles nesting near the Great Lakes or anadromous accessible rivers is at this time most likely due to the effects of PCBs and *p,p′*-DDE. Bald eagle productivity has been demonstrated to be inversely correlated with concentrations of PCBs and *p,p′*-DDE in addled eggs [8]. Greater mortality of adult bald eagles has been observed along shores of Lakes Superior, Michigan, and Erie [5,24] (P. Hunter, M. Shieldcastle, personal communication). Concentrations of PCBs in blood plasma of nestling bald eagles from Great Lakes nests were greater than from nestlings in Oregon and Washington, USA [13]. Poor reproductive success coupled with plasma concentrations of *p,p′*-DDE and PCBs greater than in unaffected subpopulations in Oregon and Washington have been noted on the Lower Columbia River [25].

Plasma from nestling bald eagles is a relative index of PCB and *p,p′*-DDE of prey within breeding territories when the results of this study are compared to concentrations in fish prey collected during the same time period [14]. The same gradient of concentrations and magnitude were observed in both eagle plasma and fish concentrations from these subpopulations [14]. As shown previously, blood can be used to measure *p,p′*-DDE in other species. A significant correlation was found between *p,p′*-DDE uptake, brain concentrations, and egg concentrations to plasma concentrations for American kestrels (*Falco sparverius*), northern goshawks (*Accipiter gentilis*), Cooper's hawks (*Accipiter cooperii*), and sharp-shinned hawks (*Accipiter striatus*) [26]. Concentrations of *p,p′*-DDE in blood serum were found to be highly correlated with concentrations in fat and breast muscle lipids of the white-faced ibis (*Plegadis chihi*) [27].

MANAGEMENT IMPLICATIONS

The importance of a vulnerable, relatively uncontaminated, forage base for bald eagle reproduction is imperative for the ability of the species to successfully reproduce. Effects of environmental contaminants on bald eagle productivity are well known [5,8,28,29]. Management techniques that control populations of eagle prey species need to take into account the effect that increases or decreases in numbers of contami-

nated species will have on bald eagle reproductive success [14]. The fact that PCBs and DDT from 1987 through 1992 were at concentrations that are associated with less than average productivities presents continuing management issues, even though production of these compounds has ceased in North America and concentrations of most halogenated hydrocarbons in the prey of eagles has decreased in the Great Lakes region [11]. Current concentrations of PCBs, *p,p′*-DDE, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents are sufficiently great to cause adverse effects in at least some birds [5,8,11,28–30]. Our results verify that productivity of eagles is inversely correlated with exposure to both PCBs and *p,p′*-DDE. A concurrent study found that productivity in the Great Lakes region was not correlated to mercury measured in feathers of adults and nestling eagles [31]. Furthermore, we have observed congenital deformities in bald eagle nestlings [32]. Developmental deformities have been observed in the populations where the greatest concentrations of PCBs have been found in the blood of nestling eagles.

Examination of our results suggests that exposure of eagles to Great Lakes fishes should be minimized. Thus, it would be premature to begin reintroduction programs to reestablish populations of eagles or improve their genetic diversity along the Great Lakes shorelines, especially Lake Erie. Furthermore, management practices that increase the potential exposure of eagles to chlorinated hydrocarbons in Great Lakes fishes, such as passage of fishes around dams on tributaries to Lakes Michigan, Huron, and Erie, could have adverse effects on productivity of bald eagles in subpopulations that currently are productive to act as a source of eagles to colonize other areas. Only by maintaining a vulnerable, relatively uncontaminated, food source for eagles during the breeding season can we continue to experience the population recovery of this species in the Midwest.

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