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CONGENER-SPECIFIC POLYCHLORINATED BIPHENYL PATTERNS IN EGGS OF AQUATIC BIRDS FROM THE LOWER LAGUNA MADRE, TEXAS

MIGUEL A. MORA*
National Biological Service, Southern Science Center, Brazos Field Station, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas, USA 77843

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Abstract—Eggs from four aquatic bird species nesting in the Lower Laguna Madre, Texas, were collected to determine differences and similarities in the accumulation of congener-specific polychlorinated biphenyls (PCBs) and to evaluate PCB impacts on reproduction. Because of the different toxicities of PCB congeners, it is important to know which congeners contribute most to total PCBs. The predominant PCB congeners were 153, 138, 180, 110, 118, 187, and 92. Collectively, congeners 153, 138, and 180 accounted for 26 to 42% of total PCBs. Congener 153 was the most abundant in Caspian terns (*Sterna caspia*) and great blue herons (*Ardea herodias*) and congener 138 was the most abundant in snowy egrets (*Egretta thula*) and tricolored herons (*Egretta tricolor*). Principal component analysis indicated a predominance of higher chlorinated biphenyls in Caspian terns and great blue herons and lower chlorinated biphenyls in tricolored herons. Snowy egrets had a predominance of pentachlorobiphenyls. These results suggest that there are differences in PCB congener patterns in closely related species and that these differences are more likely associated with the species' diet rather than metabolism. Total PCBs were significantly greater ($p < 0.05$) in Caspian terns than in the other species. Overall, PCBs in eggs of birds from the Lower Laguna Madre were below concentrations known to affect bird reproduction.

Keywords—Polychlorinated biphenyls   Congener-specific PCBs   Aquatic birds   Accumulation   Wildlife toxicology

INTRODUCTION

Congener-specific polychlorinated biphenyl (PCB) patterns in birds are not well defined. Generalities in the predominance of some PCB congeners in eggs of wild birds have been pointed out [1]; however, assessments of differences or similarities among species in the contribution by various congeners to total PCBs are still few. Because birds inhabit diverse ecosystems and feed on a variety of prey, their exposure may vary widely in degree and type of PCBs. Polychlorinated biphenyl congeners differ in their biodegradation and bioaccumulation patterns and are of different toxicities to wildlife [2]. Some known effects of PCBs include embryo deformities, edema, weight loss, and thymic atrophy [3]. Understanding differences and similarities in congener patterns among related species is important in determining differences in exposure, metabolism, or both.

Differences in congener-specific PCB patterns among organisms have been associated with differences in diet and metabolic capacities. For example, the accumulation of PCB congeners 153, 138, and 180 (IUPAC numbers) was different among herons (*Ardea cinerea*) from several locations in Great Britain and the differences in congener patterns were associated with the diets of the herons rather than metabolism [4]. Eggs of several water birds from Italy showed different congener-specific PCB patterns, which reflected differences in diets: lower chlorinated biphenyls were predominant in birds feeding on invertebrates and plant material and higher chlorinated biphenyls were predominant in fish-eating birds [5]. In contrast, Ormerod and Tyler [1] suggested that the PCB congener patterns in eggs of birds from Italy and Canada were similar and that this similarity reflected generalities in the assimilation or metabolism of PCBs by birds. In light of these apparently different results, one objective of my study was to further the understanding of congener-specific PCB patterns among related aquatic bird species and to compare my findings with those from other studies. Congeners 153, 138, and 180 are often reported as the most common in bird tissues [4,6–9]; however, more information is needed to understand differences and similarities in PCB congener patterns among species.

Polychlorinated biphenyls have been linked with embryonic abnormalities and teratogenic effects in birds [3,10]. During 1992 and 1993 two reddish egret (*Egretta rufescens*) chicks and one laughing gull (*Larus atricilla*) chick were found with deformities on the National Audubon Society Sanctuary Islands of the Lower Laguna Madre, Texas. Consequently, a second objective of my study was to determine total concentrations of PCBs in eggs of birds from the Lower Laguna Madre and to evaluate their possible detrimental effects on birds nesting in this aquatic ecosystem.

MATERIALS AND METHODS

Egg collection

Eggs were collected during 1993 from four aquatic bird species nesting on the National Audubon Sanctuary Islands of Lower Laguna Madre (Fig. 1). Eggs from tricolored herons (*Egretta tricolor*) and snowy egrets (*Egretta thula*) were collected from Green Island and eggs from great blue herons (*Ardea herodias*) and Caspian terns (*Sterna caspia*) were collected from dredge-spoil islands near Laguna Vista. Both Green Island and the dredge-spoil islands are part of the National Audubon Sanctuary Islands. Most eggs (great blue herons, tricolored herons, and snowy egrets) had recently been laid; however, eggs from Caspian terns were at intermediate or late stages of development.

* To whom correspondence may be addressed.
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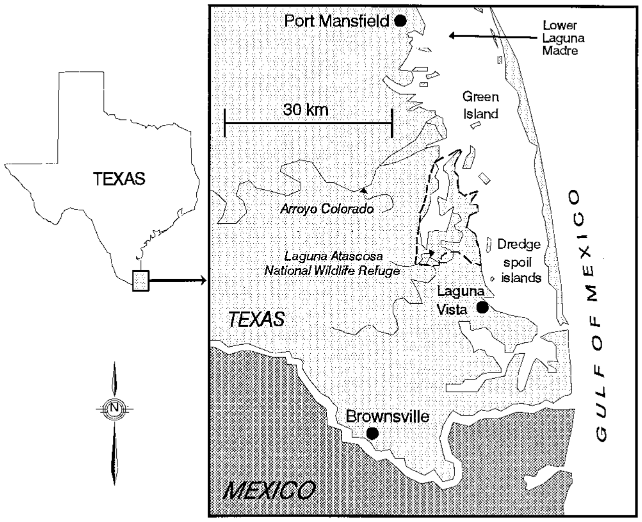

Fig. 1. Map of the Laguna Atascosa National Wildlife Refuge and National Audubon Sanctuary Islands, Lower Laguna Madre, Texas.

One egg was collected from each of 10 nests from each species for organochlorine and trace element analyses. Eggs were wrapped in aluminum foil and taken to the laboratory where the contents were transferred to chemically cleaned glass jars and stored at −20°C until chemical analysis could be performed.

Chemical analysis

Sixteen eggs (four from each species) were analyzed for congener-specific PCBs at the Geochemical and Environmental Research Group (GERG), Texas A & M University, College Station, Texas. The eggs were extracted by the NOAA status and trends method [11] with some modifications [12,13]. A portion of the egg homogenate (0.5 g) was mixed with Na_2SO_4 anhydrous and extracted with methylene chloride. The extract was then concentrated and purified with a silica gel/alumina column and then with HPLC to reduce matrix interferences. The egg extracts were analyzed with a gas chromatograph, Hewlett Packard 5880A, with split/splitless injection system, ^{63}Ni electron-capture detector, and a DB-5 (30 × 0.25 mm i.d.) fused silica capillary column (J & W Scientific). Helium was used as carrier gas. The injector and detector temperatures were 275°C and 325°C, respectively. The column temperature was programmed from 100 to 140°C at 5°C/min, then from 140 to 250°C at 1.5°C/min, and from 250 to 300°C at 10°C/min, with a holding time of 1 min at the beginning of the program and at each rate change. Eighty-seven PCB congeners were identified and quantified. Polychlorinated biphenyl congeners were confirmed by gas chromatography/mass spectrometry in 10% of the samples. Spike recoveries were above 80% in all cases; variation between duplicates was within 15%.

Statistical analysis

The PCB congener patterns were compared by principal component analysis of the 20 most common congeners with the use of procedure PRINCOMP of SAS version 6.08 software program [14]. Concentrations of congener-specific and total PCBs, the contributions of 20 congeners (explaining over 80% of the total PCB concentrations) that appeared in the four species, and congeners grouped by chlorobiphenyls were all compared by Kruskal–Wallis tests. When significant differences were observed, the Tukey multiple comparisons procedure in conjunction with ANOVA of the ranked data [14] was used to determine which means were different. The level of significance for each test was set a priori at 0.05.

Table 1. Percent moisture, percent lipids, and total PCB concentrations (mean, ng/g wet wt. ± standard error) in eggs (N = 4) of aquatic bird species from the Lower Laguna Madre, Texas

Species	Moisture	Lipid	Total PCBs
Tricolored heron (*Egretta tricolor*)	80.9 ± 0.5	4.2 ± 0.1	71 ± 18
Snowy egret (*Egretta thula*)	80.9 ± 0.6	4.5 ± 0.5	74 ± 16
Great blue heron (*Ardea herodias*)	82.1 ± 0.1	4.2 ± 0.2	246 ± 87
Caspian tern (*Sterna caspia*)	75.1 ± 0.7	6.1 ± 0.2	1,265 ± 680

RESULTS

Total PCBs were significantly greater ($p < 0.01$) in eggs of Caspian terns than in eggs of tricolored herons and snowy egrets, but were not different from eggs of great blue herons (Table 1). Polychlorinated biphenyls were similar in eggs of great blue herons, tricolored herons, and snowy egrets. Lipid percent was also greater in eggs of Caspian terns than in eggs of the other three species. Moisture percent, however, was less in Caspian terns than in the other species. Congener-specific PCB profiles for each species (percent contribution to total PCBs, $N = 4$) are shown in Figure 2. The predominant PCB congeners in the four species were 153, 138, 180, 110, 118, 187, and 92. Congener 153 was the most abundant in Caspian terns and great blue herons and congener 138 was the most abundant in snowy egrets and tricolored herons. Congeners 153, 138, and 180 contributed from 26% (snowy egrets) to 42% (great blue herons and Caspian terns) of total PCB concentrations in eggs (Fig. 3). Of the chlorobiphenyls, pentachlorobiphenyls and hexachlorobiphenyls were the most common and contributed from 61 to 79% of total PCBs (Fig. 4).

Principal component analysis of the percent contribution of individual congeners to total PCBs indicated that the first three components explained only just over 50% of the variation and five components explained 75% of the variation (Table 2). The first component explained only 25% of the variance and seemed to be a measure of the most significant contributors to total PCBs and a contrast between common and uncommon significant contributors. Thus, congeners 118, 153, 180, 187, 146, and 196 had the highest positive loadings and congeners 60, 82, 92, and 49 had the most negative loadings (Table 3). The second principal component was not as clear as the first; however, it seemed to represent a measure of the uncommon significant contributors (lower chlorinated congeners) with high positive loadings for congeners 129, 105, 110, 118, and 49 and high negative loadings for congeners 82, 92, and 99 (Table 3). Congener 118 had high loadings in both components 1 and 2. The third component was a contrast between congeners 138 and 183 with high negative loadings and the rest of the congeners.

Loadings for species sorted according to principal component 1 revealed that Caspian terns and great blue herons had the highest loadings on component 1 whereas snowy egrets and tricolored herons had the least. Sorting by the second component showed tricolored herons with the highest positive loadings followed by snowy egrets. The plot of the first two components (Fig. 5) demonstrated more clearly the distribution of species

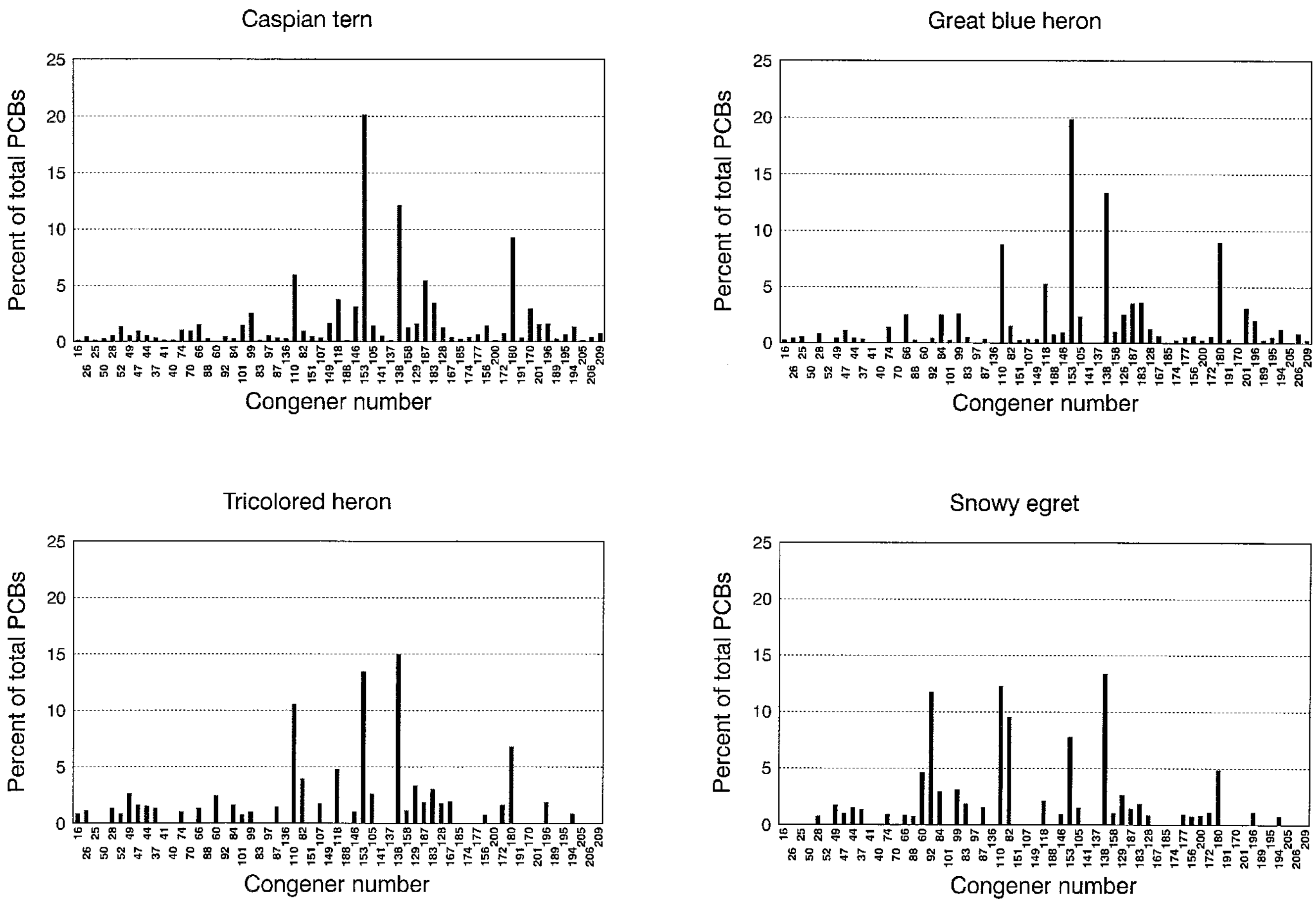

Fig. 2. Congener-specific PCB patterns (percent contribution to total PCBs) in four aquatic bird species of the Lower Laguna Madre, Texas.

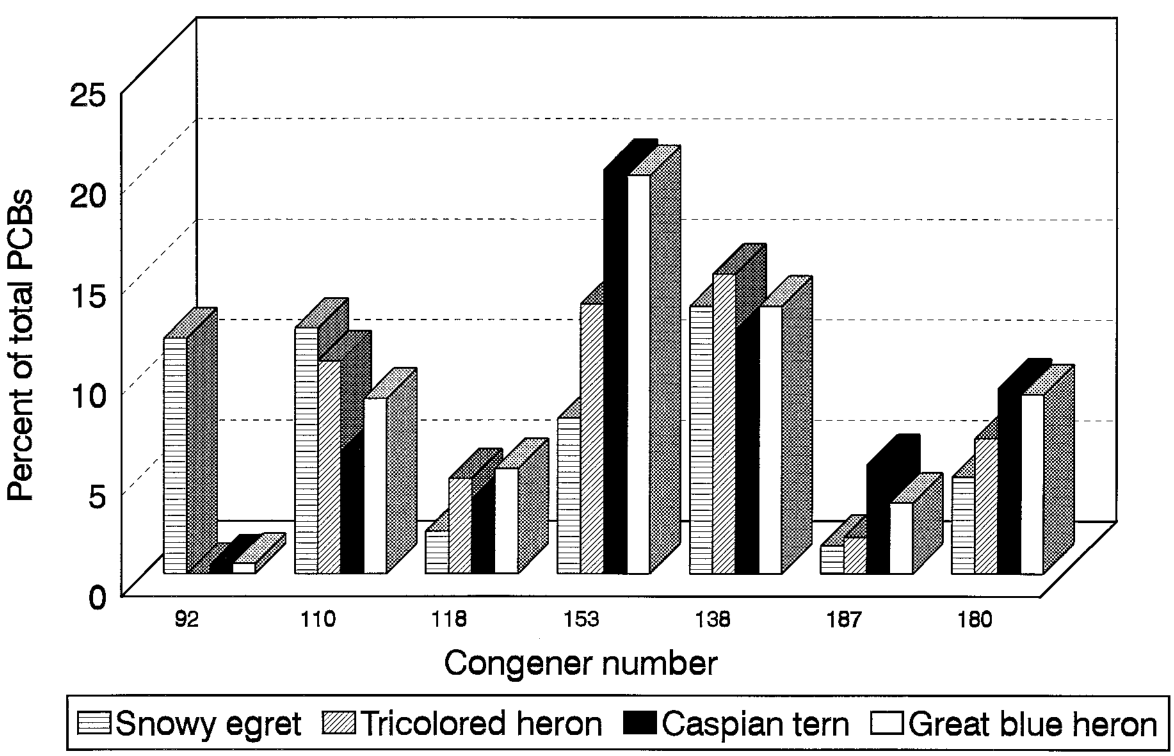

Fig. 3. Comparisons of the most common PCB congeners in aquatic birds of the Lower Laguna Madre, Texas.

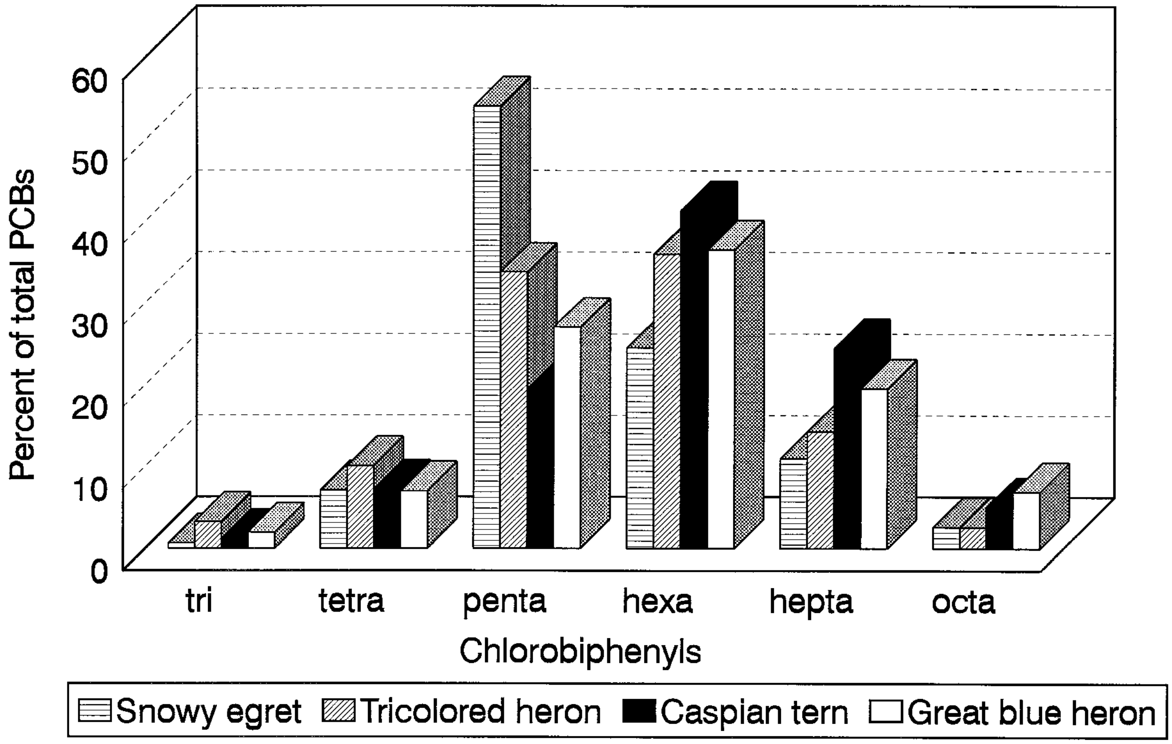

Fig. 4. Distribution of chlorobiphenyls, grouped by level of chlorination, in four aquatic bird species of the Lower Laguna Madre, Texas.

according to the first two components and showed a distinct cluster separation by species, particularly for Caspian terns and great blue herons.

Because most of the variation in PCB congener patterns among species could not be accounted by fewer than five components, the PCB congeners were grouped by level of chlorination for the principal component analysis. The results in this case were much clearer and 95% of the variation could be explained by the first three components (Table 4). The first principal component explained 54% of the variance and represented a contrast between the higher (positive loadings) and lower (negative loadings) chlorinated biphenyls (Table 5). The second component was a contrast among the lower chlorinated biphenyls with high positive loadings for trichlorobiphenyls and tetrachlorobiphenyls and negative loadings for pentachlorobiphenyls. The third principal component was represented primarily by a high loading of octachlorobiphenyls. The plot of the first two components (Fig. 6) separated out the species into distinct clusters with Caspian terns and great blue herons weighing heavily on component 1, suggesting a predominance of higher chlorinated biphenyls. The tricolored herons weighted more heavily on component 2, suggesting a predominance of lower (tri- and tetra-) chlorinated biphenyls. Snowy egrets had more negative loadings on the second component, indicating a predominance of pentachlorobiphenyls.

The results of principal component analysis were also supported by comparisons of percent contributions to total PCBs of individual congeners and chlorobiphenyl groups. In general, the contributions of 20 congeners to total PCBs were similar between the tricolored heron and snowy egret (except for congener 92), and between the Caspian tern and the great blue heron (except for congener 146). Also, the contribution of congeners 66, 82, 99, 105, 110, 118, 129, 138, 149, 153, 170, 183, and 196 to total PCBs was similar among the four species. The only significant differences among species occurred with con-

Table 2. Eigenvalues of the correlation matrix resulting from the principal component analysis of congener-specific PCBs in four aquatic bird species

Component	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	5.09	1.42	0.25	0.25
PRIN2	3.68	1.09	0.18	0.44
PRIN3	2.58	0.56	0.13	0.57
PRIN4	2.03	0.44	0.11	0.67
PRIN5	1.58	0.25	0.08	0.75

Table 3. Eigenvectors of the principal component analysis of congener-specific PCBs in four aquatic bird species

Congener	PRIN1	PRIN2	PRIN3
110	−0.12	0.27	0.34
118	0.22	0.3	−0.13
153	0.38	0.007	−0.15
105	−0.01	0.33	−0.17
138	−0.09	−0.15	−0.55
180	0.36	0.05	−0.044
187	0.39	−0.22	−0.004
183	0.16	−0.06	−0.45
146	0.29	−0.23	0.17
170	0.16	−0.12	0.22
99	−0.06	−0.29	0.26
201	0.18	0.03	0.04
82	−0.32	−0.24	−0.04
60	−0.16	−0.04	0.04
129	−0.11	0.4	−0.15
66	0.09	0.12	0.13
196	0.21	0.18	0.24
92	−0.27	−0.29	0.03
49	−0.19	0.37	0.13
149	0.17	−0.08	0.16

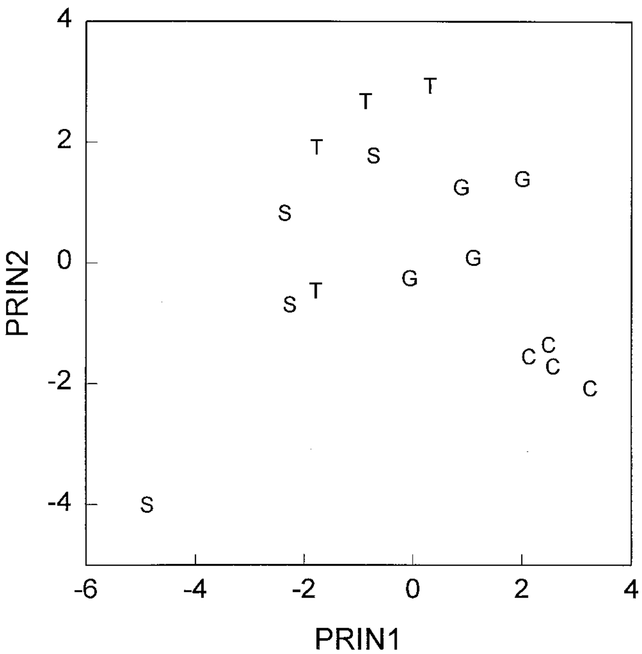

Fig. 5. Plot of principal components 1 and 2 based on principal component analysis of the contribution of 20 PCB congeners. C = Caspian tern, G = great blue heron, T = tricolored heron, S = snowy egret.

geners 49, 60, 92, 146, 180, 187, and 201. The contribution of congener 146 to total PCBs was significantly greater ($p < 0.05$) in Caspian terns than in the other three species. The contributions of congeners 187 and 201 were also significantly higher ($p < 0.001$) in Caspian terns and great blue herons than in snowy egrets and tricolored herons. The contribution of congener 180 was significantly greater ($p < 0.01$) in Caspian terns and great blue herons than in snowy egrets, but was not different from tricolored herons. The contribution of congener 92 was significantly greater ($p < 0.05$) in snowy egrets than in the other three species and congeners 49 and 60 were significantly greater ($p < 0.01$) in tricolored herons and snowy egrets than in Caspian terns and great blue herons.

Of the chlorobiphenyls, the contributions of tetrachlorobiphenyls and hexachlorobiphenyls were similar among the four species. The contribution of trichlorobiphenyls was significantly higher ($p < 0.05$) in tricolored herons than in snowy egrets and pentachlorobiphenyls were significantly higher ($p < 0.001$) in snowy egrets than in great blue herons and Caspian terns. In contrast, the contributions by heptachlorobiphenyls and octachlorobiphenyls were significantly greater ($p < 0.001$) in Caspian terns and great blue herons than in tricolored herons and snowy egrets. Overall, the contributions of chlorobiphenyls to total PCBs were similar among Caspian terns and great blue herons but seemed to be different between snowy egrets, which had a predominance of pentachlorobiphenyls, and tricolored herons, with a predominance of trichlorobiphenyls.

Table 4. Eigenvalues of the correlation matrix resulting from the principal component analysis of chlorobiphenyls in four aquatic bird species

Component	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	3.22	1.58	0.54	0.54
PRIN2	1.64	0.8	0.27	0.81
PRIN3	0.83	0.63	0.14	0.95
PRIN4	0.21	0.11	0.03	0.98
PRIN5	0.09	0.09	0.02	0.99

Table 5. Eigenvectors of the principal component analysis of chlorobiphenyls (grouped by level of chlorination) in four aquatic bird species

Chlorobiphenyl	PRIN1	PRIN2	PRIN3
Tri	−0.19	0.71	0.08
Tetra	−0.35	0.56	−0.13
Penta	−0.48	−0.38	0.13
Hexa	0.49	0.03	−0.44
Hepta	0.51	0.2	−0.06
Octa	0.32	0.11	0.87

DISCUSSION

In this study, congeners 153, 138, and 180 were the most abundant and accounted for 26 to 42% of the total PCBs. These results are similar to recent studies, which report these congeners as the most abundant in bird eggs [4–7,15,16]. Differences in contribution of congeners have been used as indicators of different species patterns. In this regard, the Caspian tern and the great blue heron were similar because they had congener 153 as the most abundant (contributing up to 20% of total PCBs); the snowy egret and tricolored heron were also similar because they had congener 138 as the most dominant. Similarly, Ormerod and Tyler [1] reported differences in PCB congener patterns of the Eurasian dipper (*Cinclus cinclus*) from several regions because congener 153 was the most common in one region and congener 118 was the most common in another. Falandysz et al. [15] found that congeners 153, 138, and 180 were the most abundant in breast muscle of the white-tailed eagle (*Haliaeetus albicilla*) and that there were differences between adults and juveniles of the same species in the contribution of congeners to total PCBs, suggesting possible different capabilities to depurate PCBs. However, the contribution of in-

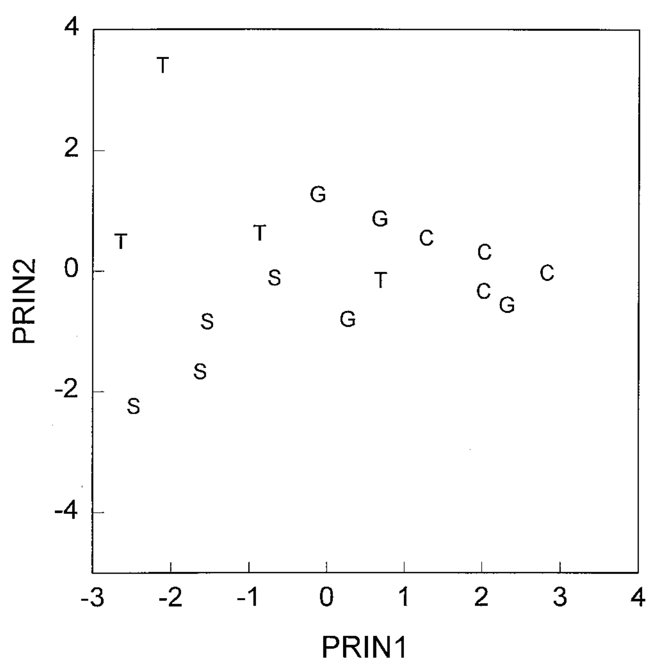

Fig. 6. Plot of principal components 1 and 2 based on principal component analysis of the contribution of PCB congeners grouped by chlorobiphenyls. Letters same as in Figure 5.

dividual congeners to total PCBs and the congener patterns of young and adult white- tailed eagles were not statistically compared, thus the apparent differences between adults and juveniles were left unproven.

The intepretation of differences in PCB congener patterns based on predominance of one compound over another should be taken with caution. A more comprehensive analysis may be needed to establish differences in congener patterns among species with certainty. For example, in this study the contributions of congeners 153 and 138 to total PCBs were not significantly different among the four species despite the fact that congener 153 was predominant in the Caspian tern and the great blue heron and congener 138 was predominant in the snowy egret and tricolored heron. Furthermore, the individual analysis of the contribution of 20 PCB congeners to total PCBs revealed that only 7 (3 of which were fairly abundant in all species) of 20 congeners were significantly different among species. The differences between the Caspian tern and the great blue heron were only in the contribution of congener 146, and between the tricolored heron and the snowy egret in the contribution of congener 92. Thus, because of the marked similarities in the two species within a group, it could be argued that the differences in congener patterns occurred only between the two species groups. The analysis of the contribution by chlorobiphenyls provided equivalent results. In this case, the differences between the two groups were clearer and were marked by a predominance of either lower or higher chlorinated biphenyls. The lower chlorinated biphenyls were more abundant in the tricolored herons, which consume more diverse diets including invertebrates and fish, whereas the higher chlorinated biphenyls were predominant in the more strictly fish-eating species or species eating larger fish, the Caspian tern and the great blue heron.

Principal component analysis was the most helpful statistical technique for elucidating differences and similarities in PCB congener patterns among species. Even though five components were necessary to explain 75% of the variance in the analysis of individual PCB congeners, the graphic representation of components 1 and 2 (Fig. 5) clearly indicated that there were species differences in congener patterns. Caspian terns and great blue herons were more positively associated with the first component, with a preponderance of higher chlorinated biphenyls, and were contrasted by the separation along the second component of the tricolored heron and snowy egret, with a greater predominance of lower chlorinated biphenyls. Further, the analysis of chlorobiphenyls showed that the distribution of species along the first and second components (Fig. 6) was similar to the individual analysis, except that now component 1 explained more than 50% of the variation and the first two components explained more than 80% of the variance. Because component 1 represented a contrast between higher and lower chlorinated biphenyls and component 2 a contrast among the lower chlorinated biphenyls, it was much easier to characterize differences in congener patterns among species based on chlorobiphenyl groups. Again, Caspian terns and great blue herons had a predominance of higher chlorinated biphenyls, snowy egrets had a predominance of pentachlorobiphenyls, and tricolored herons had a predominance of trichlorobiphenyls and tetrachlorobiphenyls.

My study supports the idea that differences in PCB congener patterns among aquatic bird species may be related to differences in congener patterns in the prey or diet. Of the species studied, the tricolored heron and snowy egret tend to feed more on invertebrates and smaller fish [17,18], whereas the great blue heron and the Caspian tern feed more on larger fish [17,19]. Invertebrates such as crustaceans or lower trophic level aquatic organisms tend to have a predominance of lower chlorinated biphenyls, whereas fish or higher trophic level organisms tend to accumulate higher chlorinated biphenyls [20,21]. Focardi et al. [5] also found that lower chlorinated compounds predominated in aquatic birds feeding on invertebrates and plant material and higher chlorinated biphenyls were more predominant in fish-eating birds. Boumphrey et al. [4] found differences in the PCB congener profiles of herons (*Ardea cinerea*) from different regions of Great Britain and suggested that the patterns were more indicative of dietary intake than of metabolism. In a similar study of organochlorine residues in various kinds of herons in the United States, residue levels were directly related to differences in the herons' diets and to the amount of fish consumed [22]. Thus, differences in the diet alone could explain differences in congener patterns among similarly related aquatic species.

Other studies, however, contrast with the above. For example, Stronkhorst et al. [23] found differences in congener patterns between the diet and bird tissues: lower chlorinated biphenyls were more abundant in the diet and higher chlorinated biphenyls were more abundant in eggs of some aquatic birds from the Netherlands. Polychlorinated biphenyl congener patterns in several bird species from Canada were similar [24], reflecting perhaps similar metabolism, because birds had diverse diets. Because eggs of Caspian terns were at a later stage of development than eggs of the other three species, the lower content of lesser chlorinated biphenyls in eggs of Caspian terns could be explained perhaps by increased embryo metabolism. However, recently laid eggs of great blue herons showed a congener pattern similar to eggs of Caspian terns, suggesting that stage of embryo development might not have an influence in the congener patterns observed in eggs. The results of my study suggest that several approaches may be necessary to distinguish differences and similarities in PCB congener signatures among related bird species. The analysis of predominance of certain congeners above others, which often has been used as the criterion to establish differences in congener patterns among related or unrelated bird species, may not be sufficient.

Concentrations of total PCBs were also different among aquatic birds of the Lower Laguna Madre and could also be explained by differences in the diet and in the amount of fish consumed. Caspian terns are strictly fish-eating birds and forage more offshore than the other species. The herons and egrets consume a broader variety of food items and forage more in salt marshes and inland lagoons [17,18]. Raptorial and fish-eating birds are more likely to accumulate chlorinated hydrocarbons at greater concentrations than other bird species [25]. An alternative explanation to the greater concentration of PCBs in eggs of Caspian terns is that these eggs were collected at a later stage of incubation than eggs of the other three species. Caspian tern eggs had greater moisture loss, which resulted in greater lipid percent and greater PCB concentrations than in eggs of the other species. Polychlorinated biphenyls, however, were 5 to 15 times higher in eggs of Caspian terns than in eggs of great blue herons, tricolored herons, and snowy egrets, whereas lipid content was only 2% higher in Caspian terns than in the other species. Thus, greater concentrations of PCBs in eggs of Caspian terns were probably reflected by greater lipid content but also by a more strictly fish diet of Capian terns than the other species.

Overall, concentrations of total PCBs in aquatic bird species nesting in the Lower Laguna Madre were low and below the threshold at which behavioral or teratogenic anomalies have been observed in other species [10,26]. Thus, based on the PCB levels observed in eggs of these four species and in reddish egrets from the same area [27], it is reasonable to conclude that PCBs were perhaps not associated with the deformities observed in two reddish egret and one laughing gull chicks during 1992 and 1993 in the Lower Laguna Madre. The results of the analysis of PCBs in tissues of these deformed specimens were very low (S. Robertson and A. Huysman, personal communication), at concentrations that could not be associated with deformities.

CONCLUSIONS

The results of this study suggest that there are differences in PCB congener patterns among closely related bird species. Whether these differences are more possibly associated with species' diet than metabolism remains to be studied. Aquatic birds with more strictly fish diets and those feeding on larger fish seem to have a predominance of higher chlorinated biphenyls, whereas birds feeding in smaller fish and a variety of other organisms including invertebrates have a predominance of lower chlorinated biphenyls. These findings are in agreement with some studies but contrast with others that report no differences in congener patterns among species. This study also supports observations from others regarding the prevalence of congeners 153, 138, and 180 in wildlife samples [4,6–9]. Congeners 138, 153, and 180 are not very susceptible to atmospheric transport and environmental degradation [9]; thus, they should be expected to predominate in wildlife species, especially those that are at the top of food chains. Because of the different toxicities of PCB congeners, it is important to know which congeners have the greatest contribution to total PCBs. Of the seven most abundant congeners in the four aquatic species in this study, the mono–ortho coplanar PCB congener 118 should be the one of most concern because of its predominance and potential increased toxicity to wildlife [28]. Overall, total PCB concentrations were low and currently do not pose a threat to aquatic birds of the Lower Laguna Madre.

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