Chlorinated Hydrocarbons and Biomarkers of Exposure in Wading Birds and Fish of the Lower Rio Grande Valley, Texas

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Abstract. During 1997 we evaluated reproductive success in colonial water birds nesting in the Lower Rio Grande Valley (LRGV), Texas, and correlated success with concentrations of contaminants in eggs. We also measured steroid hormones and gonadosomatic index (GSI) as biomarkers of endocrine effects in common carp (*Cyprinus carpio*). Nest and fledging success of green herons (*Butorides virescens*) and great egrets (*Ardea alba*) were similar to those found in other parts of North America; however, nesting success of black-crowned night-herons (*Nycticorax nycticorax*) was lower, very likely due to flooding of the nesting area. Except for DDE and toxaphene, all chlorinated pesticides in bird eggs were low and not of concern for negative effects on any of the three species. DDE was highest in green heron eggs and seemed to increase along a geographic gradient from west to east, with eggs from Falcon Reservoir containing low concentrations, and those at Los Indios containing the highest concentrations (approx. 11,000 ng/g WW), near or above the threshold for reproductive impairment. DDE levels in great egrets and black-crowned night-herons were below those that are associated with reproductive impairment. Mean DDE levels in carp at the JAS Farms site were above the threshold level suggested for predator protection. Toxaphene was detected in about 20% of the samples with high levels observed in green heron eggs from Los Indios (mean = 4,402 ng/g WW). These are the highest toxaphene levels reported in bird eggs in the LRGV. Toxaphene levels in fish ranged between 90 and 312 ng/g WW. In general, PCBs in bird eggs and fish tissue were low and at levels not of concern for reproductive effects. The greatest concentrations of testosterone and 11-ketotestosterone were detected in fish from the JAS Farms site, which also had the greatest concentrations of DDE. Increased androgen production and gonad development

in fish at this site, relative to Pharr, could be possibly associated with endocrine disrupting effects of *p,p'*-DDE. DDE, toxaphene, PCBs, and hormones were highest in birds and fish from the eastern edge of the study area.

The Lower Rio Grande Valley (LRGV) of Texas is comprised of the state's four southernmost counties, Starr, Hidalgo, Cameron, and Willacy, and encompasses roughly the lower third of the Rio Grande basin (Figure 1). Historically, this region was dominated by unique Tamaulipan thorny brush vegetation; however, today an estimated 95% of Tamaulipan brush has been cleared and replaced by agricultural, municipal, and industrial developments (Jahrsdoerfer and Leslie 1988). The LRGV is a major agricultural production area, with approximately 320,000 ha (over 75% of the geographical area) devoted to irrigated cropland (Fipps 1991). The *maquiladora* (product assembly) industry in neighboring border states in Mexico has grown substantially since the 1970s (Texas Center for Policy Studies 1996), and together with increased trade between the United States and Mexico, has caused rapid population growth in south Texas.

Much of the LRGV is actually coastal plain and Rio Grande delta, containing oxbow lakes (locally called *bancos* and *resacas*) formed from fragments of old river channels and tributaries. Originally recharged by river floodwaters, *resacas* are now filled by rainfall, runoff, or irrigation return water. Many are now used to store irrigation water pumped from the Rio Grande. Together with artificially created irrigation or settling basins, *resacas* provide a major source of freshwater habitat for LRGV wildlife (Jahrsdoerfer and Leslie 1988). Lack of regular flushing by floodwaters has caused some of these basins to become contaminant sinks (Coastal Impact Monitoring Program 1995). Several studies have indicated that the levels of contaminants such as *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), polychlorinated biphenyls (PCBs), toxaphene, mercury (Hg), and selenium (Se) are elevated in the sediments and biota at some of these sites (White *et al.* 1983;

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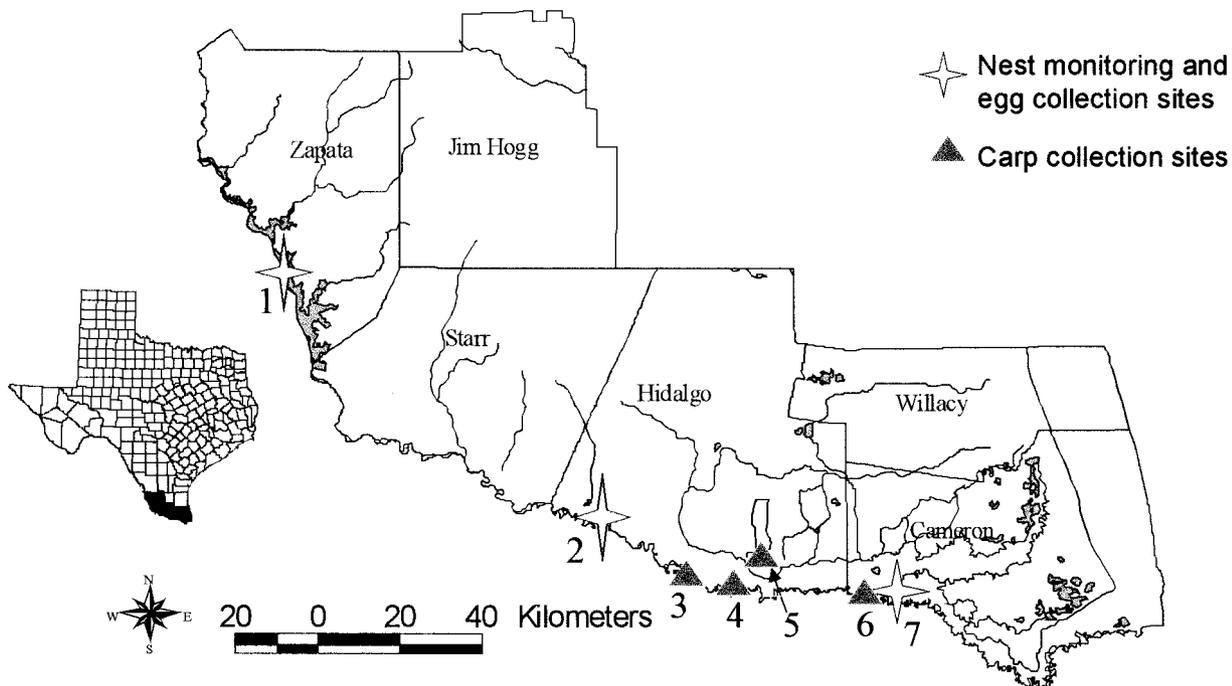
Fig. 1. Map of the Lower Rio Grande Valley showing the locations for the collection of bird eggs and fish. Numbers correspond to the following locations: 1, Falcon Reservoir; 2, Edinburg; 3, Pharr; 4, Pintail Lake Santa Ana NWR; 5, Donna Reservoir; 6, JAS Farms; 7, Los Indios

Gamble *et al.* 1988; Texas Department of Health 1993). Such studies have raised concern that chemicals associated with agriculture, increased urbanization, and industrialization may be adversely affecting wildlife using freshwater habitats in the LRGV.

Previous and recent studies have correlated contaminant exposure in fish and birds with alterations in endocrine function, such as changes in steroid hormone concentrations, protein production, and gonadal development (Fry and Toone 1981; Munkittrick *et al.* 1992; Jobling *et al.* 1996; Spies and Thomas 1997; Thomas and Khan 1997). Organics such as DDT derivatives, alkylphenols, phthalate esters, and styrenes, and metals such as cadmium and lead have been shown to have endocrine disrupting effects *in vitro* and/or *in vivo* (Colborn *et al.* 1993; Thomas and Khan 1997; Thomas 1999). Many of these compounds are products of LRGV industries and waste-water treatment facilities (Warshaw 1976; Brownsville Economic Development Council 1990). Potentially estrogenic pesticides that are currently in use on agricultural crops throughout the state of Texas include trifluralin, atrazine, carbaryl, endosulfan, parathion, synthetic pyrethroids, and aldicarb (Gianessi and Anderson 1995). Additionally, persistent pesticides with known or suspected estrogenic and antiandrogenic activity, such as DDT, dieldrin, kepone, and toxaphene, were used heavily on cotton and vegetable crops in Southern states before their use was banned in the 1970s and 1980s (Eisler and Jacknow 1985; Soto *et al.* 1994; Monosson *et al.* 1997; Loomis and Thomas 1999; Sperry and Thomas 1999).

The objectives of this study were to screen two taxonomic levels of wildlife in LRGV aquatic habitats for signs of reproductive impairment and endocrine disruption. Specifically, we evaluated reproductive success in colonial fish-eating birds as

an indicator of reproductive health and correlated success with concentrations of contaminants in eggs. One common measure of exposure and effects of environmental contaminants in birds is estimating reproductive success and correlating it with contaminants present in eggs. We also determined steroid hormones and gonadosomatic index (GSI) as biomarkers of endocrine effects in common carp (*Cyprinus carpio*). Some of the most commonly used indicators of exposure and effects of endocrine-disrupting chemicals in fish include measurements of vitellogenin and hormone levels in plasma. Common carp were selected because they were more abundant and could be obtained more easily from several locations in the LRGV.

Materials and Methods

Bird Survey and Sampling Sites

The study sites were located primarily in the southern Texas counties of Cameron and Hidalgo. One additional site was located in Zapata County (Figure 1). Four settling basins (Edinburg, Pharr, Donna, and Los Indios), two *bancos* (Pintail Lake and JAS Farms Lake), and one reservoir (Falcon) were selected as the study sites. Effort was made to select sites of each type distributed across both counties; however, it was not always possible. In general, settling basins had steep to slightly sloped edges covered in rip-rap, were typically at depths between 1.5 and 3.5 m (except during periods when more water was distributed for irrigation than was pumped in from the river), were surrounded by river cane or mowed grasses, and had little riparian vegetation. *Bancos* (oxbow lakes) had gently sloping sides, shallow depths that receded during the length of the summer, and remnant riparian vegetation and wetland vegetation along their edges.

Nest Monitoring

Small (25–30 nests in each) dispersed green heron (*Butorides virescens*) colonies were studied at two settling basins, Edinburg and Los Indios. Nests in each colony were marked and monitored weekly from April to July 1997. All nest observations were made in early morning and each monitoring session lasted about 30 min. Most nests were in trees at heights above 3–4 m and were observed using a mirror attached to an extendible pole. Number of eggs and/or chicks, estimated incubation stage or chick age, and presence or absence of adults were observed during each visit. Nests were monitored until 15 days after the first egg hatched. This was considered the fledging date. If a nest failed before the fledge date, cause of failure (*i.e.*, depredation, failure to hatch, abandonment, etc.) was determined if possible. Failed nests were checked for reuse during each monitoring session.

Two larger colonies containing nests of great egrets (*Ardea alba*), cattle egrets (*Bubulcus ibis*), snowy egrets (*Egretta thula*), black-crowned night-herons (*Nycticorax nycticorax*), green herons, little blue herons (*E. caerulea*), tricolored herons (*E. tricolor*), neotropic cormorants (*Phalacrocorax brasilianus*), and anhingas (*Anhinga anhinga*) were monitored at Falcon Reservoir, the westernmost sampling location in the LRGV. Nests of black-crowned night-heron, green herons, and great egrets were checked five times between 18 May and 15 July 1997 by boat, using the methods indicated above.

Nest initiation, hatch, and termination dates were calculated for all species based on an incubation period of 22 days for green herons (Davis and Kushlan 1994) and 25 days for great egrets and black-crowned night-herons (Harrison 1978; Davis 1993). A fledging date of 15 days was established for nests of each species as described above.

Sample Collection and Tissue Preparation

At each colony one fresh green heron egg was collected by hand from three nests. Additionally, at Falcon one fresh egg was collected from each of nine monitored great egret and black-crowned night-heron nests. Four unhatched great egret eggs, each from a different nest, also were collected for contaminant analyses. Nests were reached by extendible or freestanding ladders or by boat, and eggs were collected only from complete clutches. Eggs were placed immediately on wet ice for transport, wrapped in aluminum foil, and refrigerated less than 3 h after collection, and processed less than 24 h after collection. Egg contents were poured into chemically cleaned glass jars. If an embryo was present, it was evaluated for gross abnormalities and developmental stage. Samples were stored at $-25°C$ immediately after processing.

Male common carp were collected by gill net from two settling basins (Donna, n = 4, and Pharr, n = 4), and two *bancos* (Pintail Lake, n = 2, and JAS Farms, n = 3) (Figure 1) in July and August 1997. These sites were selected as representative of settling basins and oxbow lakes in the LRGV. An effort was made to collect fish of similar sizes and in the same reproductive stage. Sex and reproductive stage of all fish were confirmed by histological examination of gonad tissue. Total length (length from tip of snout to longest point of caudal fin) and weight of each fish were recorded and each fish was examined for signs of external and internal lesions and parasites. Immediately on collection, blood was drawn from the caudal vein using a heparinized needle and syringe. Blood was transferred to a 5-ml Vacutainer containing sodium heparin and stored on wet ice for less than 3 h until taken to the laboratory. In the laboratory, whole blood was centrifuged to separate plasma, which was stored in cryovials at $-25°C$. Gonads were weighted and gonadal somatic index (GSI) was calculated by dividing gonad weight by somatic weight and multiplying by 100. Whole fish were then wrapped in aluminum foil and stored at $-25°C$.

Extraction and Residue Analysis

Egg contents were homogenized in their original chemically cleaned container using a hand-held tissumizer blender. Frozen whole fish were sliced using a band saw and then ground in a Hobart grinder until thoroughly homogenized. The chemical analyses were conducted at the Geochemical and Environmental Research Laboratory, Texas A&M University, with procedures described in MacLeod *et al.* (1985) with a few modifications (Sericano 1993). Approximately 2 g of avian egg homogenate and 10 g of whole carp homogenate were used for the analysis of selected chlorinated pesticides and PCBs. After the addition of 50 g of anhydrous Na_2SO_4, the tissue was extracted three times with 100 ml of methylene chloride using a tissumizer homogenizer. The organic phase was concentrated to 10–15 ml and then heated in Kuderna-Danish tubes to a final volume of 2 ml in hexane.

Egg and carp extracts were fractionated by alumina:silica (80–100 mesh) column chromatography. Activated copper was added to the top of the column to remove any residue of elemental sulfur in the egg extracts. The resulting fraction was concentrated as previously described and further purified by high-pressure liquid chromatography (HPLC) to remove any excess lipid materials. The extract from HPLC was concentrated to a final volume of approximately 1 ml in hexane for gas chromatographic/electron capture detection.

PCBs and chlorinated pesticides were analyzed by fused-silica capillary column GC-ECD (Ni[63]) using a Hewlett Packard 5890 Series II gas chromatograph in the splitless mode. Tetrachloro-m-xylene (TCMX) was used as the GC internal standard to calculate the recoveries of the surrogates. Mean recoveries of surrogate standards 4,4'-dibromoocto-fluorobiphenyl (DBOFB), PCB 103, and PCB 198, were 72.4%, 62.1%, and 60.7%, respectively, for carp samples, and 74.6%, 76.4%, and 76.5%, respectively, for bird eggs. The detection limits for 23 chlorinated pesticides and 21 PCB congeners averaged 0.34 ng/g and 0.46 ng/g, respectively, for 2-g sample sizes. A total of 94 PCB congeners were analyzed. A group of PCB congeners (8, 18, 28, 44, 52, 66, 101, 105, 110, 118, 128, 138, 153, 170, 180, 187, 195, 206, and 209) were quantitated against a set of authentic standards, which were injected at four different known concentrations to calibrate the instrument and to compensate for nonlinear response of the electron capture detector. The remaining PCB congeners were quantitated by comparison to a single reference congener of the same degree of chlorination injected at four different known concentrations. Contaminants were identified and quantified using X-Chrom software. Extractable lipids in egg and carp samples were determined from 20-ml aliquots of the sample extracts that were allowed to evaporate until dry. The residue was redissolved in 1 ml of methylene chloride, 0.1 ml was placed on filter paper and evaporated, and the residual materials were weighed using a Cahn 29 electrobalance. Moisture content of each sample was determined from a 1-g subsample of homogenized tissue. The subsample was dried in an oven at 50°C for at least 24 h or until constant weight.

Toxaphene Extraction

Further extraction procedures were necessary to isolate toxaphene from PCBs in green heron and common carp samples from diverse locations. For each sample, 500 μl of the extract obtained in the above method were spiked with 50 μl ε-hexachlorocyclohexane (ε-HCH) as a surrogate standard. The extracts were fractionated by silica column chromatography. After collection of the first fraction containing PCB congeners and some chlorinated pesticides, 100 ml of 1:1 methylene chloride:pentane were applied to the column and a second fraction containing toxaphene and most other chlorinated pesticides was collected. Separated fractions were concentrated to 10–15 ml, and then heated in Kuderna-Danish tubes to concentrate the extracts to a final volume of 0.5 ml in hexane. Samples were analyzed for toxaphene by fused-silica capillary column GC-ECD as described above. Concentration of total toxaphene was calculated based

on 11 toxaphene peaks measured in toxaphene calibration standards of four different known concentrations.

Determination of Steroid Hormone Concentrations in Carp Plasma

Plasma testosterone (T) and 11-ketotestosterone (11-KT) content in male carp were determined by radioimmunoassay (RIA) at University of Texas Marine Science Institute, Port Aransas, Texas, with procedures previously described (Smith and Thomas 1991). For each assay, quality controls consisted of duplicate solvent blanks and a set of blank and spiked pooled plasma. Recovery of steroid internal standard (40–250 pg/tube) ranged from 83.0% to 90.6% for T and 73.4% to 84.4% for 11-KT. Steroid hormone concentrations were calculated based on the standard curve using Beckman ImmunoFit EIA/RIA Analysis software, version 3 (Beckman Instruments, Palo Alto, CA).

Statistical Analyses

Nest success and fledging success were compared among sites and species by two-tailed z-tests using CONTRAST software (Hines and Sauer 1989). Results for chlorinated pesticides and PCBs are expressed on a ng/g WW basis, and for hormones on a ng/ml basis. Normality of residual variance was determined for all variables based on the Shapiro-Wilks statistic (W, $p > 0.05$) computed by the univariate procedure in SAS (SAS Institute Inc. 1987). Concentrations were square root or logarithmically transformed to adjust for normality, therefore, geometric means are reported. When a contaminant was detected in at least 50% of the samples, a value equal to one-half of the minimum detection limit was assigned to samples in which the contaminant was not detected. Site and species differences in contaminant means in bird eggs and carp were tested by analysis of variance using the general linear model procedure in SAS (SAS Institute Inc. 1987). Tukey's HSD test, adjusted for unequal sample sizes among treatment groups (Zar 1984), was used to compare means. Toxaphene concentrations were compared by t tests. The Pearson correlation procedure (SAS Institute Inc. 1987) was used to determine relationships among plasma steroids, GSI, length, weight, and contaminant concentrations in male common carp.

Results

Avian Reproductive Success

Table 1 shows the general results of our observations of nesting and fledging success of herons and egrets. Mean clutch size, hatching, and fledging success were similar among green herons from Los Indios and Edinburg in the LRGV (two-tailed Z-test $p < 0.05$). Green herons from Falcon were not included because of small sample size. Clutch size was lower for black-crowned night-herons than for great egrets or green herons. The number of nests with hatchlings, eggs hatched per nest, percent fledging, and number of young fledged per nest were similar between green herons and great egrets; however, they were 2.5 to 4 times lower in black-crowned night-herons than in green herons or great egrets. Nine of the 13 black-crowned night-herons nests observed were destroyed by flooding, thus black-crowned night-herons were affected by this event more than the other species.

Contaminants in Avian Eggs

Geometric means and ranges of chlorinated hydrocarbons found in greater than 50% (except toxaphene) of all egg samples are reported in Table 2. Except for DDE, concentrations of most chlorinated pesticides were low and near the limits of detection. Mean DDE concentrations ranged from 119 ng/g WW in green herons from Falcon Reservoir to near 11,000 ng/g WW in green herons from Los Indios in Cameron County. Toxaphene was present at relatively high values (1,058–4,402 ng/g WW) in green heron eggs from Los Indios and Edinburg. In almost all instances, pesticide concentrations were greatest in green heron eggs from Los Indios and lowest in green heron eggs from Falcon Reservoir. Green heron eggs from Los Indios had significantly higher ($p < 0.05$) *p,p'*-DDE concentrations than eggs from all other sites and species.

Concentrations of chlorinated pesticides were not significantly different between eggs of black-crowned night-herons and great egrets from Falcon Reservoir, nor were they different from concentrations of contaminants in green heron eggs from Edinburg in Hidalgo County.

Individual PCB congeners and total PCBs (Table 2) were generally low and were not significantly different among species and sites. However, PCB concentrations in great egret, black-crowned night-heron, and Los Indios green heron eggs were at least four times higher than total PCB concentrations in green heron eggs from Falcon or Edinburg.

Contaminants in Fish

Male common carp were caught in sufficient numbers for contaminant analyses at Donna Reservoir, Pharr Settling Basin, JAS Farms *banco*, and Pintail Lake. Male common carp are considered mature at a length of 310 mm and females at 375 mm (Goodbred *et al.* 1997). All male carp at Donna, JAS Farms, and Pharr were considered mature based on this criterion, and mean length was not significantly different (adjusted Tukey's HSD, $p > 0.05$) among male carp from these sites. Male carp from Pintail Lake had a mean length of 260 mm and were considered mature because their gonads contained sperm. Mean lengths of carp from Donna, JAS Farms, and Pharr, however, were significantly greater ($p < 0.05$) than the mean length of Pintail Lake carp.

Geometric means and ranges of chlorinated pesticides and total PCBs detected in ≥50% of carp are presented in Table 3. One carp at both Donna and Pharr and two carp from JAS Farms showed small, unresolved complex mixtures, and these samples were further extracted for toxaphene. Fish from JAS Farms had the greatest concentrations of all pesticides, including DDE and toxaphene, whereas concentrations in fish from Pintail Lake were much lower than in fish from other sites. Similar to bird eggs, all chlorinated pesticides were detected at very low concentrations in fish, except for DDE and toxaphene. These two compounds were significantly greater in fish from JAS Farms than in fish from any other location. Geometric means for total PCBs did not differ significantly among carp from Donna, JAS, and Pharr; however, carp from Pintail Lake had significantly lower total PCBs than fish from any of the former three sites ($p < 0.05$).

Table 1. Nest and fledging success of green herons, great egrets, and black-crowned night-herons in the LRGV, Texas

Species and Site	Nests Observed	Clutch Size (mean ± SD)	Nests with Hatchlings (%)	Eggs Hatched/Nest (mean ± SD)	Nests with Fledglings (%)	Young Fledged/Nest (mean ± SD)
Green heron						
Los Indios	17	3.85 ± 0.90	13 (76.5)	2.50 ± 1.70	8 (61.5)	2.00 ± 1.87
Edinburg	17	4.00 ± 0.67	13 (76.5)	2.40 ± 1.51	10 (76.9)	2.33 ± 2.08
Falcon	4	4.2 ± 0.45	3 (60)	2.00 ± 1.63	3 (100)	1.92 ± 1.63
Black-crowned night-heron						
Falcon	13	2.91 ± 1.22	4 (31)	0.55 ± 1.29	3 (75)	0.45 ± 1.04
Great egret						
Falcon	28	3.22 ± 0.75	22 (78.6)	2.36 ± 1.32	17 (77)	1.78 ± 1.45

Table 2. Geometric mean concentrations and ranges (ng/g, WW) of chlorinated pesticides and total PCBs in bird eggs from the LRGV, Texas

Compound	% Detect[a]	Great Egret Falcon Res 13[b]	Black-Crowned Night-Heron Falcon Res 9	Green Heron Falcon Res 3	Edinburg 3	Los Indios 3
Moisture %		82 (80–84)	82 (81–82)	81 (80–82)	82 (81–82)	82 (80–82)
Lipid %		4.5 (2.8–6.5)	4.9 (3.8–5.7)	4.5 (3.4–5.9)	3.9 (3.2–4.6)	4.8 (3.9–5.5)
trans-Nonachlor	100	9 (3–149)	7 (3–29)	3 (2–5)	9 (5–14)	23 (12–46)
Dieldrin	100	7 (2–300)	4 (1–13)	1 (0.9–3)	20 (11–29)	76 (37–138)
Toxaphene	19.4	ND[c]	ND	ND	1,058 (177–2,961)	4,402 (3,157–7,870)
p,p'-DDD	93.5	7 (3–33)	7 (1–16)	NC[d]	13 (7–21)	21 (9–51)
p,p'-DDE	100	1,085 B (369–4,701)	1,480 B (562–4,876)	119 C (64–275)	2,421 B (2,133–2,683)	10,954 A (6,925–19,377)
p,p'-DDT	93.5	3 (1–105)	4 (1–48)	0.4 (ND–0.6)	21 (8–36)	19 (16–22)
Total PCBs	100	265 A (82–1,498)	264 A (92–659)	57 A (48–74)	73 A (50–146)	219 A (130–320)

Values sharing the same letters within rows (DDE and PCBs only) are not significantly different (adjusted Tukey HSD, $p < 0.05$)
[a] Percent of total eggs with detectable levels
[b] Number of eggs analyzed from each species at each site
[c] ND = Not detected
[d] NC = Not calculated

Endocrine Biomarkers in Fish

All male common carp from JAS Farms and all but one from Pharr were in the same reproductive stage (most common gonad stage was spermatid). Gonads from carp at Donna were not evaluated for reproductive stage. These fish had enlarged gonads when collected, but may have been in an earlier reproductive stage than fish collected from Pharr and JAS Farms because they were collected a month earlier. Gonads of male carp from Pintail Lake contained sperm and therefore were not in the same stage as fish from other sites. Table 4 shows morphometric and biomarker measurements in common carp. Total length and body weight were similar among carp from Donna, JAS, and Pharr, and were significantly higher than in fish from Pintail Lake. Gonad weight and gonadosomatic index were significantly higher in carp from JAS Farms than in carp from Donna and Pintail Lake. Plasma testosterone and 11-ketotestosterone levels were significantly higher in carp from JAS than in carp from the other three sites which were similar.

Table 5 shows a Pearson correlation matrix of contaminants and biomarker values in carp. Gonad weight was significantly correlated to testosterone, 11-ketotestosterone, PCBs, and DDE. Gonadosomatic index was significantly correlated to testosterone, 11-ketotestosterone, and DDE, but not to PCBs. Testosterone was significantly correlated to gonad weight, GSI, 11-ketotestosterone, and DDE, but not to total PCBs. Mean 11-ketotestosterone was significantly correlated to gonad weight, GSI, testosterone, and DDE, but not to PCBs. DDE was significantly correlated to all the morphometric measurements and hormone levels in plasma. PCBs were only correlated to DDE and gonad weight.

Table 3. Geometric means and ranges (ng/g, WW) of chlorinated pesticides and total PCBs in male common carp from the LRGV, Texas

Pesticide	Donna 4[a]	JAS 3	Pintail Lake 4	Pharr 5
Moisture %	74 (73–76)	69 (65–72)	76 (72–78)	74 (71–77)
Lipid %	2.5 (1.5–3.9)	7.4 (5.9–9.1)	4.5 (2.8–7.8)	3.3 (1.6–5.2)
trans-Nonachlor	2 (2–4)	4 (2–7)	0.6 (0.4–0.9)	5 (2–9)
Dieldrin	2 (2–3)	7 (4–11)	1 (0.8–1.4)	1 (1–2)
Toxaphene	164[b] B	296 A (281–312)	ND[c]	91[b]
p,p'-DDE	170 B (76–487)	1,085 A (355–2,117)	67 B (59–83)	351 B (99–752)
p,p'-DDD	4 (3–9)	89 (20–201)	2 (1–2)	7 (4–13)
p,p'-DDT	NC[d]	2 (1.6–2.4)	ND	NC
Total PCBs	94 A (62–201)	123 A (65–220)	29 B (25–34)	82 A (44–123)

Values sharing the same letters within rows (DDE, toxaphene, and PCBs) are not significantly different.
[a] Number of samples.
[b] Only one sample from each of these sites was analyzed for toxaphene.
[c] ND = Not detected.
[d] NC = Not calculated.

Table 4. Geometric means and ranges of male common carp morphometric measurements and biomarkers

	Donna 4[a]	JAS 3	Pintail Lake 2	Pharr 4
Total length (mm)	398 A (384–422)	490 A (370–625)	260 B (255–265)	463 A (462–490)
Total weight (g)	822 A (760–940)	1,380 A (680–1,460)	259 B (250–268)	1,169 A (1,010–1,280)
Gonad weight (g)	4.7 BC (2.8–14.2)	69.7 A (65.3–125.3)	1.3 C (0.3–5.2)	34.2 AB (18.5–53.3)
Gonadosomatic index (g)	0.63 BC (0.38–1.54)	5.29 A (4.68–6.46)	0.094 C (0.046–1.92)	1.77 AB (1.61–4.49)
Testosterone (ng/ml)	1.29 B (0.77–2.21)	6.68 A (4.89–9.03)	1.02 B (0.56–1.86)	1.69 B (0.68–3.23)
11-ketotestosterone (ng/ml)	0.88 B (0.52–2.22)	6.24 A (6.21–6.29)	1.16 B (0.83–2.06)	1.94 B (1.48–2.30)

Values sharing the same letters within rows are not significantly different.
[a] Number of samples analyzed.

Discussion

Avian Reproductive Success

At all three study sites green heron nest success and fledging success (about 75%) were similar to those found in other studies. For instance, green herons in San Blas, Mexico, had a hatching success of 65.5% (Dickerman and Gavino 1969), whereas green herons in New York (Meyerriecks 1962) and Missouri (Kaiser and Reid 1987) had approximately 79% hatching success. Hatching success of green herons at a contaminant area in the Yazoo National Wildlife Refuge in Mississippi was 88%, however, hatching success decreased signif-

icantly in nests with eggs containing DDE residues greater than 5,100 ng/g WW (White *et al.* 1988).

Black-crowned night-heron nest success in the LRGV was about 45% lower than that reported at various sites in San Francisco Bay, California, where it ranged from 47% to 70%, (Hothem *et al.* 1995). Mean DDE concentrations in night-herons from San Francisco Bay ranged from 500 to 6,000 ng/g WW. At a Lake Ontario night-heron colony where eggs had high pesticide residues, hatching success ranged between 36% and 54% over a 4-year span (Price 1977). Night-heron nest success at Falcon was low due to destruction of nests by flooding.

Nest and fledging success of great egrets was generally similar to that of great egrets at Sapelo Island, Georgia, during

Table 5. Pearson correlation (r) matrix of morphometric, biomarker, and contaminant values of male common carp from selected locations in the LRGV, Texas

	Gonad Weight	Gonadosomatic Index	Testosterone	11-Keto-testosterone	PCBs	DDE
Gonad weight	1.0	0.858***	0.541*	0.746***	0.522*	0.697*
Gonadosomatic index	0.858***	1.0	0.496*	0.748***	0.267	0.572**
Testosterone	0.541*	0.496*	1.0	0.731**	0.367	0.618**
11-keto-testosterone	0.746***	0.748***	0.731**	1.0	0.216	0.736**
PCBs	0.522*	0.267	0.367	0.216	1.0	0.854***
DDE	0.697*	0.572**	0.618**	0.736**	0.854***	1.0

*** $p < 0.001$
** $p < 0.01$
* $p < 0.05$

1958, where 94.5% of eggs hatched (Teal 1965). Fledging success of great egrets in this study (77%), was greater than that found over a 4-year study at a contaminated colony in Audubon Canyon Ranch, California (28–52%, Pratt 1972). At this site, eggshell breakage was deemed the most important factor in egg loss. Contaminant analyses revealed DDE concentrations as high as 300,000 ng/g in egg yolk, and 124,300 ng/g WW in livers of adult great egrets (Pratt 1972).

Nest and fledging success for all species except night-herons were similar to those found in other studies. The nesting success information collected in our study suggests that egrets and herons nesting in the LRGV are reproducing well; however, sublethal effects of persistent bioaccumulative toxicants, particularly DDE, may still occur. Monitoring of a greater number of nests and analysis of more samples would better indicate whether current DDE levels, particularly in green herons, are likely to cause negative effects on reproduction of aquatic birds in the LRGV.

Contaminants in Avian Eggs

Except DDE, and toxaphene to some extent, all chlorinated pesticides in green herons, black-crowned night-herons, and great egrets were low and not of concern for negative effects on these species. Contaminants in green heron eggs seemed to increase along a geographic gradient from west to east, with eggs from Falcon Reservoir containing low concentrations of all contaminants, those at Edinburg containing an intermediate level, and those at Los Indios containing the highest contaminant values. This may be explained perhaps by increased agricultural practices in the eastern part of the LRGV, which would result in more use of pesticides and more contaminated aquatic habitats than in the western side of the valley. At Falcon and Edinburg, mean DDE was well below concentrations (~ 5,100 ng/g) suggested to cause reproductive impairment in green herons (White *et al.* 1988), whereas at Los Indios the mean DDE concentration (10,954 ng/g) was higher than the above threshold. The three eggs collected from Los Indios contained 6,925, 9,794, and 19,377 ng/g DDE. In each of these nests the remaining eggs hatched; however, chicks from the nest with the highest DDE concentration were found dead before fledging. Unfortunately, the dead chicks were too decomposed to be analyzed for DDE.

DDE also was the pesticide that occurred at the greatest concentration in all night-heron and great egret eggs at Falcon. Mean DDE concentrations in night-heron eggs were below the level, 8,000 ng/g, associated with reproductive impairment (Henny *et al.* 1984). Shell breakage, egg failure, and shell thinning were observed in great egret eggs with DDE levels of 300,000 ng/g ww (Pratt 1972). This value is well beyond the threshold for reproductive effects in many birds (Blus 1996). For example, DDE levels of 3,000 ng/g in great blue heron (*Ardea herodias*) eggs have been associated with reduced hatching (Blus 1996). Great egret eggs in our study had mean DDE levels lower than 3,000 ng/g.

Potential sources of DDE in bird eggs at the two LRGV sites and at Falcon are probably local from heavy use of DDT in the past, but also could have been accumulated on wintering grounds. Although adult green herons are known to be migratory, populations in Southern states are thought to be resident (Davis and Kushlan 1994). Thus, it is possible that DDE, at least in green herons, comes from local sources. DDE concentrations in green heron eggs from Los Indios were only slightly lower than those found in carcasses of laughing gulls and Forster's terns (*Sterna forsteri*) feeding in a nearby agricultural drain in 1978 (White *et al.* 1983). This suggests that DDE is still present in this environment at high concentrations. Current DDE values in waterbird eggs from the Laguna Madre of eastern Cameron County, an area that receives agricultural drainwater from the LRGV, are below 1,000 ng/g and have decreased steadily since the late 1970s (White *et al.* 1984; Custer and Mitchell 1987; Mora 1996a). No previous studies on contaminants and reproduction in fish-eating birds nesting in Hidalgo and western Cameron Counties are available; thus, it is difficult to ascertain if DDE concentrations in breeding birds occupying high trophic levels in this area have declined.

At Falcon Reservoir mean DDE values were higher in black-crowned night-herons and great egrets than in green herons, in agreement with findings from other areas in the eastern United States (Ohlendorf *et al.* 1979). Such a pattern suggests that night-herons and great egrets may be feeding on more contaminated or larger prey or that they acquired their residues in different areas. Green heron diets are composed mainly of fish with a median length of approximately 55 mm (Davis and Kushlan 1994). Great egrets and night-herons may take a variety of prey items, including amphibians, crustaceans, mammals, and large fish (Davis 1993; Stokes and Stokes 1996). These larger prey items may have greater concentrations of contaminants because they have longer life spans in which to

accumulate lipophilic contaminants or they may feed at a higher trophic level than green heron prey. Levels of total DDT in sediments at Falcon have remained constant at about 5,000 ng/g since the early 1970s and indicate a potential continuous source of the pesticide to this system (Van Metre *et al.* 1997).

Dieldrin concentrations were generally low in all three species of birds; however, one great egret egg contained 300 ng/g dieldrin and one green heron egg from Los Indios contained 138 ng/g. Dieldrin is generally associated with reproductive effects in birds only at much higher levels (16,000 ng/g and up) (Peakall 1996), but this contaminant is of interest because it has recently been identified as estrogenic in mammalian cells (Soto *et al.* 1994). Exposure to dieldrin during embryonic development may have the potential to alter sexual development in wildlife.

Toxaphene was found in green heron eggs and carp from LRGV sites. Toxaphene is a broad-spectrum pesticide composed of a mixture of over 250 polychlorinated monoterpenes in use in the United States between 1947 and 1986 (Sergeant and Onuska 1995). Toxaphene continues to be a pesticide of concern because of its persistence and propensity for atmospheric transport. It is often found in high concentrations in regions where it has never been used, such as the Arctic (Paasivirta and Rantio 1991). This pesticide was used extensively in Texas and other Southern states mainly on cotton, but also on vegetable, corn, soybean, and peanut crops (Sergeant and Onuska 1995; Pearson *et al.* 1997). During 1978–79 White *et al.* (1983) found toxaphene concentrations as high as 29,600 ng/g in gizzard shad (*Dorosoma cepedianum*) and laughing gulls (3,000 ng/g) from the Arroyo Colorado, a major agricultural drain in the LRGV. Gamble *et al.* (1988) reported toxaphene values as high as 20,000 ng/g in sediments, 5,100 ng/g in fish (unidentified sp), and 7,100 ng/g in spiny softshell turtles (*Trionyx spiniferus*) from LRGV sites. In our study, green heron eggs from Edinburg had mean toxaphene concentrations of 1,085 ng/g with a maximum of 2,961 ng/g, whereas eggs from Los Indios had a mean concentration of 4,402 ng/g with a maximum value of 7,870 ng/g. These are the highest reported values of toxaphene in LRGV birds.

In general, toxaphene is not considered highly toxic to birds because most avian species can readily metabolize and excrete this pesticide (Eisler and Jacknow 1985). Niethammer *et al.* (1984) showed that green herons in Louisiana oxbow lake systems accumulated toxaphene to levels as high as 24,000 ng/g but reported no negative effects at these levels. American black ducks (*Anas rubripes*) fed either 10,000 or 50,000 ng/g toxaphene for 19 months showed no significant differences in survival, egg production, fertility, hatchability, eggshell thickness, or growth and survival of chicks compared to controls (Haseltine *et al.* 1980). Carcasses of adult ducks in the feeding study all contained less than 500 ng/g at the end of the study. Ring-necked pheasants (*Phasianus colchicus*) fed 300,000 ng/g toxaphene did exhibit reproductive effects, such as decreased clutch size and egg hatchability (Pollock and Kilgore 1978).

It has been suggested that toxaphene is prevalent enough in the LRGV ecosystem for some species to develop resistance to it (Andreasen 1985). Mosquitofish (*Gambusia affinis*) collected in the LRGV were 122 times more resistant to toxaphene than control fish. Lower Rio Grande mosquitofish survived longer and accumulated more toxaphene than other mosquitofish, thus it may have important implications for biomagnification of

toxaphene throughout the LRGV food web. Mosquitofish are within the prey size range of green herons (Davis and Kushlan 1994) and may play a part in the presence of high levels of toxaphene in these birds. New concerns have been raised over toxaphene because it has been identified as an estrogenic substance in human cell lines. Soto *et al.* (1994) found that a 10 μM (4,800 ng/g) dose of toxaphene induced estrogenic effects in human breast cancer cells. This concentration is well within the range of those found in green heron eggs from Los Indios in the LRGV. It is currently unknown whether toxaphene acts as an environmental estrogen in birds. If so, exposure of the embryo to toxaphene via maternal transport to the egg has the potential to cause abnormalities in sexual development.

Concentrations of total PCBs in eggs of all three bird species at all sites were much lower than levels (>4,000 ng/g) shown to cause reduced hatching, embryo mortality, and deformities in birds (Hoffman *et al.* 1996). The highest mean values of PCBs reported in LRGV birds were 4,000 ng/g in laughing gull carcasses collected at the mouth of the Arroyo Colorado, and 4,000 ng/g in pied-billed grebe (*Podilymbus podiceps*) in the Arroyo Colorado west of the mouth in 1978 (White *et al.* 1983). Levels of PCBs in LRGV birds have declined since that time (White *et al.* 1985; Mora 1996b). PCB values in this study support previous findings of declines and suggest that industrial inputs of PCBs into the LRGV ecosystem are not great.

Heavy metals and metalloids were not analyzed in bird eggs in this study. However, some of the metals, particularly mercury and selenium, have been pointed out of concern for potential negative effects on birds from the LRGV (Mora and Wainwright 1998).

Contaminants in Fish

Chlorinated pesticide levels in common carp were similar to pesticide levels found over the past decade in various studies of LRGV carp (Mora and Wainwright 1997). At JAS, mean DDE concentrations were above 1,000 ng/g, the threshold level for predator protection (Texas Natural Resources Conservation Commission 1991) and could pose a threat to fish-eating birds and snakes. Toxaphene was found in individual fish at Donna, JAS, and Pharr at levels ranging from 91 to 312 ng/g, which were similar to concentrations in LRGV carp samples collected in the early to mid-1980s when toxaphene was still in limited use (Texas Department of Health 1995). Fish species occupying higher trophic levels than carp that were collected during the same 1980s surveys had toxaphene levels between 1,000 and 6,400 ng/g.

Toxaphene is more detrimental to fish and aquatic invertebrates than to birds or other wildlife (Eisler and Jacknow 1985). In brook trout (*Salvelinus fontinalis*), whole body levels of 400 ng/g were associated with reductions in growth, abnormal bone development, and reduced fecundity (Mayer and Mehrle 1977). Too few samples were available in this study to establish trends in toxaphene at different sites or to determine the relationship between toxaphene and biomarkers of estrogenic exposure.

Whole body burdens of total PCBs in carp were lower than those associated with egg and fry mortality, adult mortality, adult length and weight, and hepatic lesions in a variety of fish

species (Niimi 1996). In lake trout induction of aryl hydrocarbon hydroxylase activity, a biochemical marker of PCB exposure, was positively correlated with total PCB levels similar to those in LRGV carp (Luxon *et al.* 1987). Overall, PCBs in carp were lower than those reported in LRGV fish in previous years (Mora and Wainwright 1997). This was especially true of carp from Donna, a known hot spot of PCB contamination. In 1993 the Texas Department of Health issued a consumption advisory of fish from Donna and its surrounding canals after elevated levels of PCBs (1,000–24,000 ng/g) were found in carp and smallmouth buffalo (*Ictiobus bubalus*) fillets sampled from this site (Buchanan 1997).

Hormones in Carp and Their Relationship to Contaminants

Under normal circumstances gonadal recrudescence (increased gametogenic activity) in male carp is accompanied by an increase in the plasma androgens, testosterone and 11-ketotestosterone, which are important in differentiation of spermatogonia and spermatogenesis. Once male carp begin to spawn, there is a steroid shift from androgen production to progestin production, and androgen levels decline rapidly (Barry *et al.* 1990). Carp in this study were collected during July (Donna site) and August to measure plasma steroids during recrudescence. Carp from JAS Farms had the highest levels of testosterone and 11-KT, as well as the highest GSI values out of all carp collected. Carp from Pharr, which were the same size and collected at the same time as JAS Farms males, had significantly lower levels of testosterone, 11-KT, and lower GSIs. Moreover, GSIs and plasma testosterone levels were also greater in female carp collected from JAS Farms than in those collected from the other sites (unpublished data). One possible explanation for these site differences is that the high body burdens of *p,p'*-DDE in JAS carp antagonize negative-feedback regulation of gonadotropin secretion by androgens, causing a rise in gonadotropin levels which would result in increased androgen production and gonad development (Sperry and Thomas 1999; Monosson *et al.* 1997; Khan *et al.* 1999). Interestingly, increased androgen production has also been observed in ovaries from kelp bass with high body burdens of *p,p'*-DDE collected from the Southern California Bight (Spies and Thomas 1997). However, the evidence for endocrine disruption associated with a high tissue concentration of *p,p'*-DDE should be considered preliminary, due to the relatively small number of samples collected in the present study. Additional sampling during different stages of the reproductive cycle will be required to confirm there is a clear relationship between *p,p'*-DDE bioaccumulation and increased plasma androgen levels and gonadal development in certain carp populations in the LRGV.

Conclusions

Concentrations of chlorinated contaminants (DDE, toxaphene, PCBs) were highest in birds and fish at the eastern edge of the study area. DDE is still found in some portions of the LRGV at relatively high levels that could possibly affect reproduction

and the endocrine system of birds, fish, and other wildlife. At Pharr, carp had lower plasma androgen levels and GSI than carp at the JAS Farms. More fish samples from Pharr and JAS Farms should be studied to determine more conclusively if endocrine impairment is occurring at the JAS Farms relative to the Pharr site. Future studies in the LRGV also should consider not only chlorinated compounds but other classes of xenobiotics that may have endocrine-disrupting properties, such as metals, surfactants, and organophosphorous and carbamate insecticides. The limited information available from this study suggests that contamination follows a regional distribution, rather than a pattern based on impoundment type. In general, chlorinated contaminants levels increased toward the eastern end of the LRGV agricultural area, and may reflect localized sources or drainage patterns.

References

Andreasen JK (1985) Insecticide resistance in mosquitofish of the Lower Rio Grande Valley of Texas—an ecological hazard? Arch Environ Contam Toxicol 14:573–577

Barry TP, Santos AJG, Furukawa K, Aida K, Hanyu I (1990) Steroid profiles during spawning in male common carp. Gen Comp Endocrinol 80:223–231

Blus LJ (1996) DDT, DDD, and DDE in birds. In: Beyer NW, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife—interpreting tissue concentrations. Lewis Publishers, Boca Raton, FL, pp 49–71

Brownsville Economic Development Council (1990) Directory of manufacturers and processors. Brownsville, TX, 43 pp

Buchanan TA (1997) Polychlorinated biphenyls in fish in the Donna Reservoir and Canal System and in the Lower Rio Grande Valley of Texas. M.S. thesis, University of Texas, School of Public Health, Houston, TX, 86 pp

Coastal Impact Monitoring Program (1995) Report of literature review on discharges from the Rio Grande and Arroyo Colorado and their impacts. Texas General Land Office (94-099R), Austin, TX, 79 pp

Colborn T, vom Saal FS, Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384

Custer TW, Mitchell CA (1987) Organochlorine contaminants and reproductive success of black skimmers in South Texas, 1984. J Field Ornithol 58:480–489

Davis WE (1993) Black-crowned night-heron (*Nycticorax nycticorax*). In: Poole A, Gill F (eds) The birds of North America, No. 74. The American Ornithologists' Union, Washington, DC, 20 pp

Davis WE, Kushlan JA (1994) Green heron (*Butorides virescens*). In: Poole A, Gill F (eds) The birds of North America, No. 129. The American Ornithologists' Union, Washington, DC, 24 pp

Dickerman RW, Gavino G (1969) Studies of a nesting colony of green herons at San Blas, Nayarit, Mexico. Living Bird 8:95–111

Eisler R, Jacknow J (1985) Toxaphene hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish Wildl Serv Biol Rep 85(1.4), 26 pp

Fipps G (1991) Irrigation drainage water quality issues in the Lower Rio Grande Valley of Texas. In: Byrne J, Fleming C (eds) Proceedings of IRDC-90: irrigation, research, and development in the 1990s. July 17–20, University of Lethbridge, Water Resources Institute, Lethbridge, AB, Canada, pp 463–468

Fry DM, Toone CK (1981) DDT-induced feminization of gull embryos. Science 213:922–924

Gamble LR, Jackson G, Maurer TC (1988) Organochlorine, trace element, and petroleum hydrocarbon contaminants investigation of the Lower Rio Grande Valley, Texas, 1985–1986. US Fish and Wildlife Service, Ecological Services, Corpus Christi, TX, 34 pp

Gianessi LP, Anderson JE (1995) Pesticide use in Texas crop production. National Center for Food and Agriculture Policy, Washington, DC, 74 pp

Goodbred SL, Gilliom RJ, Gross TS, Denslow NP, Bryant WL, Schoeb TR (1997) Reconnaissance of 17β-estradiol, 11-ketotestosterone, vitellogenin, and gonad histopathology in common carp of United States streams: potential for contaminant-induced endocrine disruption. US Geological Survey, Open-File Report 96-627, Sacramento, CA, 47 pp

Harrison C (1978) Field guide to the nests, eggs and nestlings of North American birds. William Collins Sons & Co Ltd, Glasgow, England, 416 pp

Haseltine SD, Finley MT, Cromartie E (1980) Reproduction and residue accumulation in black ducks fed toxaphene. Arch Environ Contam Toxicol 9:461–471

Henny CJ, Blus LJ, Krynitsky AJ, Bunck CM (1984) Current impact of DDE on black-crowned night-herons in the intermountain west. J Wildl Manage 48:1–13

Hines JE, Sauer JR (1989) Program CONTRAST—a general program for the analysis of several survival or recovery rate estimates. US Fish Wildl Serv Fish Wildl Tech Rep 24:1–7

Hoffman DJ, Rice CP, Kubiak TJ (1996) PCBs and dioxins in birds. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife—interpreting tissue concentrations. Lewis Publishers, Boca Raton, FL, pp 165–207

Hothem RL, Roster DL, King KA, Keldsen TJ, Marois KC, Wainwright SE (1995) Spatial and temporal trends of contaminants in eggs of wading birds from San Francisco Bay, California. Environ Toxicol Chem 14:1319–1331

Jahrsdoerfer SE, Leslie DM (1988) Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: description, human impacts, and management options. US Fish Wildl Serv Biol Rep 88(36), 61 pp

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP (1996) Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ Toxicol Chem 15:194–202

Kaiser MS, Reid FA (1987) A comparison of green-backed heron nesting in two freshwater ecosystems. Colon Waterbirds 10:78–83

Khan IA, Hawkins MB, Thomas P (1999) Gonadal-stage dependent effects of gonadal steroids on gonadotropin II secretion in the Atlantic croaker (*Micropogonias undulatus*). Biol Reprod 61:834–841

Loomis K, Thomas P (1999) Binding characteristics of estrogen receptor (ER) in Atlantic croaker (*Micropogonias undulatus*) testis: a different affinity for estrogens and xenobiotics from that of hepatic ER. Biol Reprod 61:51–60

Luxon PL, Hodson PV, Borgmann U (1987) Hepatic aryl hydroxylase activity of lake trout (*Salvelinus namaycush*) as an indicator of organic pollution. Environ Toxicol Chem 6:649–657

MacLeod WD, Brown DW, Friedman AJ, Burrows DG, Maynes O, Pearce RW, Wigren CA, Bogar RG (1985) Standard analytical procedures of the NOAA National Analytical Facility, 1985–1986: extractable toxic organic components, 2nd ed. US Dept of Commerce, NOAA/NMFS, NOAA Tech Memo NMFS F/NWC-92, 121 pp

Mayer FL Jr, Mehrle PM (1977) Toxicological aspects of toxaphene in fish: a summary. Trans N Am Wildl Nat Res Conf 42:365–373

Meyerriecks AJ (1962) Green heron. In: Palmer RS (ed) Handbook of North American birds, Vol. 1. Yale University Press, New Haven, CT, pp 419–426

Monosson E, Kelce WR, Mac M, Gray LE (1997) Environmental antiandrogens: potential effects on fish reproduction and development. In: Rolland R, Gilbertson M, Peterson RE (eds) Chemically induced alterations in functional development and reproduction of fishes. Proceedings from a session at the Wingspread Conference Center; 21–23 July 1995, Racine, WI. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, pp 53–60

Mora MA (1996a) Organochlorines and trace elements in four colonial waterbird species nesting in the Lower Laguna Madre, Texas. Arch Environ Contam Toxicol 31:533–537

Mora MA (1996b) Congener-specific polychlorinated biphenyl patterns in eggs of aquatic birds from the Lower Laguna Madre, Texas. Environ Toxicol Chem 15:1003–1010

Mora MA, Wainwright SE (1997) Environmental contaminants in biota of the Rio Grande/Rio Bravo basin: a review of status and trends. Final report prepared for the Biomonitoring for Environmental Status and Trends (BEST) Program, Biol Res Div, US Geological Survey, Dept of Interior, 291 pp

Mora MA, Wainwright SE (1998) DDE, mercury, and selenium in biota, sediments, and water of the Rio Grande–Rio Bravo Basin, 1965–1995. Rev Environ Contam Toxicol 158:1–52

Munkittrick KR, Van Der Kraak GJ, McMaster ME, Portt CB (1992) Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown. Environ Toxicol Chem 11:1427–1439

Niethammer KR, White DH, Baskett TS, Sayre MW (1984) Presence and biomagnification of organochlorine chemical residues in oxbow lakes of northeastern Louisiana. Arch Environ Contam Toxicol 13:63–74

Niimi AJ (1996) PCBs in aquatic organisms. In: Beyer NW, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife—interpreting tissue concentrations. Lewis Publishers, Boca Raton, FL, pp 117–152

Ohlendorf HM, Klaas EE, Kaiser TE (1979) Environmental pollutants and eggshell thickness: aninghas and wading birds in the eastern United States. US Fish and Wildlife Service, Special Scientific Report 216, Washington, DC, pp 1–94

Paasivirta J, Rantio T (1991) Chloroterpenes and other organochlorines in Baltic, Finnish and Arctic wildlife. Chemosphere 22:47–55

Peakall DB (1996) Dieldrin and other cyclodiene pesticides in wildlife. In: Beyer NW, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife—interpreting tissue concentrations. Lewis Publishers, Boca Raton, FL, pp 73–97

Pearson RF, Swackhamer DL, Eisenreich SJ, Long DT (1997) Concentrations, accumulations, and inventories of toxaphene in sediments of the Great Lakes. Environ Sci Technol 31:3523–3529

Pollock GA, Kilgore WW (1978) Toxaphene. Residue Rev 69:87–140

Pratt HM (1972) Nesting success of common egrets and great blue herons in the San Francisco Bay region. Condor 74:447–453

Price IM (1977) Environmental contaminants in relation to Canadian wildlife. Trans N Amer Wildl Nat Res Conf, Washington, DC, pp 382–396

SAS Institute (1987) SAS/STAT guide for personal computers, version 6 edition. SAS Institute, Cary, NC, 1,028 pp

Sergeant DB, Onuska FI (1995) Analysis of toxaphene in environmental samples. In: Afghan BK, Chau ASY (eds) Analysis of trace

organics in the aquatic environment. CRC Press, Boca Raton, FL, pp 69–118

Sericano JL (1993) The American oyster (*Crassostrea virginica*) as a bioindicator of trace organic contamination. Ph.D. dissertation, Texas A&M University, 242 pp

Smith JS, Thomas P (1991) Changes in hepatic estrogen-receptor concentrations during the annual reproductive and ovarian cycles of a marine teleost, the spotted seatrout, *Cyanoscion nebulosus*. Gen Comp Endocrinol 81:234–245

Soto AM, Chung KL, Sonnenschein C (1994) The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect 102:380–383

Sperry TS, Thomas P (1999) Identification of two nuclear androgen receptors in kelp bass (*Paralabrax clathratus*) and their binding affinities for xenobiotics: comparison with Atlantic croaker (*Micropogonias undulatus*) androgen receptors. Biol Reprod 61:1152–1161

Spies RB, Thomas P (1997) Reproductive and endocrine status of female kelp bass from a contaminated site in the Southern California Bight and estrogen receptor binding of DDTs. In: Rolland RM, Gilbertson M, Peterson RE (eds) Chemically induced alterations in functional development and reproduction of fishes. SETAC Technical Publication Series, pp 113–133

Stokes D, Stokes L (1996) Stokes field guide to birds, eastern region. Little, Brown and Company, Boston, MA, 471 pp

Teal JM (1965) Nesting success of egrets and herons in Georgia. Wilson Bull 77:257–263

Texas Center for Policy Studies (1996) The maquiladora boom is on! Ambiente Fronterizo, March, pp 11–12

Texas Department of Health (1993) Health and water agencies call news conference on PCBs in the Valley. News Release, 24 June 1993, Austin, TX

Texas Department of Health (1995) Fish tissue sampling data 1980–1993. Austin, TX, 224 pp

Texas Natural Resource Conservation Commission (1991) Texas surface water quality standards. Austin, TX, 138 pp

Thomas P (1999) Nontraditional sites of endocrine disruption by chemicals on the hypothalamus-pituitary-gonadal axis: interactions with steroid membrane receptors, monoaminergic pathways and signal transduction systems. In: Naz RK (ed) Endocrine disruptors: effects on male and female reproductive systems. CRC Press, Boca Raton, FL, pp 3–38

Thomas P, Khan IA (1997) Mechanisms of chemical interference with reproductive endocrine function in sciaenid fishes. In: Rolland R, Gilbertson M, Peterson RE (eds) Chemically induced alterations in functional development and reproduction of fishes. Proceedings from a session at the Wingspread Conference Center; 21–23 July 1995, Racine, WI. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, pp 29–51

Van Metre PC, Mahler BJ, Callender E (1997) Water-quality trends in the Rio Grande/Rio Bravo Basin using sediment cores from reservoirs. US Dept of the Interior, US Geological Survey Fact Sheet FS-221-96, 8 pp

Warshaw S (1976) Metal concentrations in water and sediment of Texas. Texas Water Quality Board, Special Report SR-4, Austin, TX, 31 pp

White DH, Fleming WJ, Ensor KL (1988) Pesticide contamination and hatching success of waterbirds in Mississippi. J Wildl Manage 52:724–729

White DH, Mitchell CA, Kennedy HD, Krynitsky AJ, Ribick MA (1983) Elevated DDE and toxaphene residues in fishes and birds reflect local contamination in the Lower Rio Grande Valley, Texas. Southw Nat 28:325–333

White DH, Mitchell CA, Stafford CJ (1985) Organochlorine concentrations, whole body weights, and lipid content of black skimmers wintering in Mexico and in South Texas, 1983. Bull Environ Contam Toxicol 34:513–517

White DH, Mitchell CA, Swineford DM (1984) Reproductive success of black skimmers in Texas relative to environmental pollutants. J Field Ornithol 55:18–30

Zar JH (1984) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ, 781 pp