

SEX RATIO SKEW AND BREEDING PATTERNS OF GULLS: DEMOGRAPHIC AND TOXICOLOGICAL CONSIDERATIONS

D. MICHAEL FRY,¹ C. KUEHLER TOONE,^{1,2} STEVEN M. SPEICH,^{3,4} AND
R. JOHN PEARD³

ABSTRACT.—Female-female pairing and nests with supernormal clutches (SNC) of eggs occur in gull breeding colonies when two conditions are met: (1) sex ratio of breeding adults is skewed to females; and (2) nest sites are available for female-female pairs or polygynous trios. Expanding gull populations forming new colonies have SNC arising presumably from greater dispersion of females from their natal site. Declining populations in areas polluted with organochlorines (OC) show a decrease in breeding males and up to 15% of SNC. Population change has been predictive of both SNC incidence and sex ratio skew. SNC frequency and historical occurrence parallel high levels of OC pollution in southern California and the Great Lakes. OC exposure comparable to southern California has been experimentally duplicated by injecting OC into gull eggs, causing abnormal development of both male and female embryos. Males are feminized with germ cells located in the cortex of the gonad. Females develop both right and left oviducts. Persistent right oviducts, eggshell thinning and SNC in populations of gulls in Puget Sound, WA indicate recent exposure to estrogenic pollutants.

Female-female pairing in gulls was first described in southern California by Hunt and Hunt (1973, 1977) after examination of the sexes of birds attending supernormal clutches (SNC) of four to six eggs. Subsequent studies have documented SNC, female-female pairing, and polygynous trios among breeding populations of 4 species of gulls in North America: Western Gulls (*Larus occidentalis*) in small colonies on the Channel Islands in southern California; Herring Gulls (*Larus argentatus*) breeding in areas polluted with organochlorine pollutants in the Great Lakes; Ring-billed Gulls (*Larus delawarensis*) breeding in both polluted and unpolluted areas of the Great Lakes; and Ring-billed Gulls and California Gulls (*Larus californicus*) breeding in small colonies in Washington and Idaho (see review by Conover 1984a).

Ring-billed Gulls have been undergoing a rapid range and population expansion on islands in the Great Lakes (Ludwig 1974, Fox and Boersma 1983, Blokpoel and Tessier 1986) and in the Pacific Northwest (Conover et al. 1979b), and in both regions colonies have been described with female-female pairs (Fox and Boersma 1983, Ryder and Somppi 1979, Kovacs and Ryder 1981, 1983, Conover et al. 1979a). The analysis by Fox and Boersma (1983) indicates that female-female pairing in Ring-billed Gulls correlates with newly formed or rapidly expanding breeding colonies and that supernormal clutches are rare in stable

colonies. The overall breeding population of California Gulls in the Pacific Northwest is similarly expanding (Conover et al. 1979b), and California Gull colonies with female-female pairing and SNC are similar to Ring-billed Gull colonies in that the birds move from year to year with changing conditions on islands in rivers.

The demographics of Western and Herring gull colonies with female-female pairs are markedly different from those of California and Ring-billed gulls. Colonies with SNC have been located in areas of high organochlorine contamination, and the breeding population of several of these colonies has decreased over the period from 1960-1975. Supernormal clutches of Western Gulls were first described by Schreiber (1970) and subsequently by Harper (1971), Hunt and Hunt (1973), Gress (1974), and Hand (1980). The Santa Barbara Island colony had an usually high incidence of supernormal clutches with as many as 14% of nests being attended by 2 females. The breeding population of Santa Barbara Island was severely impacted by organochlorine pollutants in the decade prior to the period of highest incidence of female-female pairing, and the breeding population of the colony declined from 3000 birds in 1972 to 850 in 1978 (Sowls et al. 1980).

Herring Gull colonies nesting in isolated areas of the Great Lakes also have had a high incidence of supernormal clutches and female-female pairing (Shugart 1980, Fitch and Shugart 1983). Reduced reproductive success of Herring Gulls occurred in colonies on Lake Michigan and Lake Ontario contaminated with organochlorine pollutants in the 1960s and early 1970s (Keith 1966, Hickey and Anderson 1968, Gilman et al. 1977, Mineau et al. 1984) and breeding success began to increase in parallel with reduced pollution during the late 1970s (Fitch and Shugart 1983,

¹ Department of Avian Sciences, University of California, Davis, California 95616.

² Present address: San Diego Zoo, Balboa Park, San Diego, California 92112.

³ Cascadia Research Collective, Olympia, Washington 98501.

⁴ Present address: P.O. Box 260-E, Malheur Field Stn, Princeton, Oregon 97721.

Mineau et al. 1984). The demographic parallels with Western Gulls are striking, as incidence of supernormal clutches or polygynous trios correlates with high organochlorine contamination, both geographically and temporally. Both species have had a marked increase in frequency of SNC since 1950, paralleling the use of organochlorine insecticides and the extent of organochlorine contamination in both southern California and the Great Lakes. Furthermore, stable breeding colonies of Western and Herring gulls in less polluted areas have not exhibited SNC or female-female pairing (Pierotti 1981, Burger and Gochfeld 1981, Nisbet and Drury 1984, Spear et al. this symposium).

The common factors in colonies of all 4 species of gulls with supernormal clutches appear to be the presence of a large surplus of breeding age females plus availability of suitable nest sites within the colonies. Nest site availability is covered in the discussion. Fox and Boersma (1983) and Fetterolf et al. (1984) postulated that new gull colonies formed during range expansion or colonization contain an excess of females due to greater dispersal of females from their natal sites. Male Herring Gulls are more philopatric than females (Chabrzyk and Coulson 1976) and thus, if other species of gull are similar, new colonies or colonies experiencing an influx of birds would be expected to have an excess of breeding age females (Greenwood 1980). Fox and Boersma (1983) concluded that SNCs result from behavioral responses to a skewed operational sex ratio accompanying range expansion and/or colonization.

Further evidence that female-female pairing is a result of a sex ratio skew is the experimental work of Conover and Hunt (1984a) who altered the sex ratio by trapping and removing adult male gulls from small Ring-billed and California gull colonies, thus creating an excess of females. The incidence of supernormal clutches and female-female pairing increased in these experimentally manipulated colonies, supporting the hypothesis that sex ratio skew encourages female-female pairing. The number of female-female pairs induced by artificial manipulation of the sex ratio was, however, considerably lower than the number of males removed, indicating that additional factors may play important roles such as nest-site availability (see discussion) and preference for male mates.

If sex ratio skew is a primary factor favoring the formation of female-female pairs and polygynous trios, an explanation for the cause of a large surplus of females in populations of Herring (Shugart 1980) and Western gulls (Hunt et al. 1980) must be found. The association of high levels of DDT in southern California, a decreas-

ing breeding population of gulls, and a high proportion of female-female pairs were central to the hypothesis of Fry and Toone (1981) that organochlorines were involved with the abnormal sex ratios in these declining populations.

Gulls are moderately resistant to effects of DDT on eggshell thinning (Peakall 1975), and levels of total-DDT which caused severe eggshell thinning in pelicans and cormorants in southern California did not severely damage egg shells of gulls. Gull chicks in this region were hatched from eggs contaminated by relatively high concentrations of organochlorines. Keith (1966) described high levels of t-DDT in Herring Gull eggs in colonies on Lake Michigan where embryos survived concentrations as high as 200 ppm (fresh-egg weight basis). Direct embryo toxicity was observed at higher levels, but the reproductive tracts of exposed embryos were never examined.

While adult gulls are resistant to egg-shell thinning caused by organochlorines, gull embryos are quite sensitive to the teratogenic effects of estrogenic substances, which include several organochlorine pollutants and metabolites. The teratogenicity of estrogens to gulls was demonstrated by Boss (1943) and Boss and Witschi (1947) by injection of the synthetic estrogen, diethylstilbestrol (DES)(3,4-bis(*p*-hydroxyphenyl)-3-hexane), into Herring Gull eggs. Concentrations of 2.5 µg per egg (equivalent to 25 parts per billion in a 100 g egg), resulted in alteration of development and partial feminization of the reproductive tracts of males. The changes found in the reproductive tracts included development of ovarian cortical tissue in the testes, incorporation of primordial germ cells in the abnormal cortex of the gonad, and persistence of oviducts. Several estrogenic organochlorines have been identified including DDT and methoxychlor (Lutz-Ostertag and David 1973, review by Kupper and Bulger 1980, Fry and Toone 1981), kepone (Eroschenko and Palmiter 1980, Eroschenko 1981), and mirex, which is metabolized to kepone (Eroschenko and Palmiter 1980). The estrogenic potential of DDT and high levels of DDT in gull eggs prompted Fry and Toone (1981) to speculate that the reproductive failures and sex ratio skew of breeding populations of Western Gulls could be a result of pollutant caused abnormal embryonic development.

We describe here results of injections of organochlorines (o,p'-DDT, p,p'-DDT, p,p'-DDE, and methoxychlor) into fertile eggs of Western and California gulls and the effects on survival and development of the reproductive system. In addition we present evidence that colonies of Glaucous-winged Gulls (*Larus glaucescens*) breeding in localized polluted areas of Puget Sound, WA, have exhibited egg shell thinning,

persistent right oviducts in adult females, and a high frequency of supernormal clutches consistent with the hypothesis of organochlorine-induced abnormal development.

METHODS

INJECTION OF GULL EGGS AND EMBRYO DEVELOPMENT

Collection of eggs and injection of organochlorines and estradiol

We obtained 317 eggs from two colonies: the Western Gull colony on Southeast Farallon Island (SEFI), CA, and the California Gull colony at Mono Lake, CA. Ninety-seven eggs were collected from SEFI in 1979, 110 collected from SEFI in 1980, and 110 eggs were collected from Mono Lake in 1980. One egg each from incomplete clutches were collected to maximize the probability that unincubated eggs were being collected. Eggs were packed in foam rubber in cardboard cases and transported to U.C. Davis within 12 hours of collection and stored at 10°C for 4 to 6 hours prior to injection and incubation.

Egg injections

Four organochlorines were injected into eggs. All were commercial compounds recrystallized by the Department of Environmental Toxicology, U.C. Davis, prior to use. Compounds injected were p,p'-DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane), Aldrich Chemical Co. "gold seal"; p,p'-DDE (2,2-bis(4-chlorophenyl) 1,1-dichloroethylene), Aldrich, 99%; o,p'-DDT (1-(2-chlorophenyl)1-(4-chlorophenyl)-2,2,2-trichloroethane), Aldrich "gold seal"; and methoxychlor (1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane), City Chemical Corp. Compounds were injected at levels of 2, 5, 20, 50, and 100 µg/g egg wt. (fresh-egg weight basis), reflecting the range of pollutant concentrations found in pelican and gull eggs in southern California in 1970 and 1973. The estrogenic positive control, 17β-estradiol benzoate (1,3,5(10)-estratrien-3,17-diol 3-benzoate), Sigma Chemical Co., was injected in eggs at 0.5, 2, 5, and 20 ppm (fresh-egg weight basis). Compounds for injection were dissolved in corn oil and injected directly into the yolk. Total volume injected was 0.5% of total egg weight (92–104 g). Injection holes were covered with cellophane tape which prevented albumen leakage and entry of bacteria and fungi. Direct yolk injection was selected so as to mimic the distribution of fat-soluble pollutants in eggs. We successfully injected 264 eggs from which 108 embryos survived and developed to pipping. Control eggs were injected with corn oil alone. Yolks of 53 eggs were damaged during injection and excluded from the results.

Incubation program

Eggs were incubated in Marsh Farms incubators at 37°C and 50–55% relative humidity. Incubation period for California Gulls was 26 days; that for Western Gulls was 28 days. Chicks were removed from eggs and killed at hatching and examined for gross lesions. The reproductive tracts were photographed *in situ*, and gonads were fixed in buffered 10% formalin or 4% glutaral-

dehyde for histology. Tissues were embedded in paraffin or water-soluble plastic (Sorvall JB-4), sectioned, and stained with hematoxylin-eosin or basic fuchsin-methylene blue. Serial sections of all left gonads were cut transverse to the long axis of the gonad, and each section was scored for the presence of primordial germ cells (PGC) in the cortex of the gonad. Testes and ovaries were sectioned and positive identification of testes of extensively feminized males was made from the presence of seminiferous tubules in the medullary portion of the gonad. Data from both species were pooled for analysis as no differences in the sensitivity of embryos or extent of developmental differences were observed between the 2 species. Fisher's Exact Test was used to determine the significance of differences between treatment groups (Zar 1984).

SURVEY OF GLAUCCOUS-WINGED GULLS IN PUGET SOUND

Glauccous-winged Gulls were studied in northern and western Washington as part of a 1984 National Oceanic and Atmospheric Administration (NOAA) survey of the population health of marine mammals and birds (Calambokidis et al. 1985). Gulls were collected from 5 breeding colonies: Goose Island in Gray's Harbor in western Washington; Smith Island in the Straits of Juan de Fuca 12 km west of Whidbey Island; Smith Cove at the north end of the Seattle waterfront; the St. Regis Paper Company, Tacoma; and the Simpson Timber Company, Shelton, at the southwestern end of Puget Sound. Two sites (Goose and Smith islands) were chosen as breeding colonies in relatively unpolluted areas and three sites (Seattle, Tacoma, and Shelton) were unusual colonies on roof tops and on piers in industrial areas and were selected on the basis of their historical or potential pollution impacts. The Seattle colony consists of several nesting locations on pilings, docks, and rooftops of warehouses and financial district offices (reviewed by Eddy 1982). The number of birds has grown slowly since 4 birds were first observed in 1936. Nearly 230 pairs nested on pier warehouses in 1981, but many of the warehouses were removed in 1982–1984 and the colony may therefore be nest-site limited at present (Eddy, pers. comm.). Surveys of the 5 breeding colonies were made during May, June, and July, 1984 (3–11 censuses per colony), during peak incubation.

Adult incubating females and their clutches of 3 eggs were collected during the first third of incubation. Adults were trapped on their nests and were weighed and measured to determine sex. In these colonies all birds weighing less than 1000 g were females, making separation easy. Females trapped on their nests were carried alive to the necropsy area. Blood was taken from the brachial vein for hematological studies, and the birds were killed with an inhalation overdose of ethyl ether. Gross necropsies were performed in the field, tissues were taken for residue analysis, and all remaining internal organs were fixed in 10% buffered formalin for histological examination. Complete gross and histopathological analyses were compiled for each bird from each study site and are presented elsewhere (Calambokidis et al. 1985).

All collected eggs were blown to remove contents which were retained frozen for residue analysis. Egg-

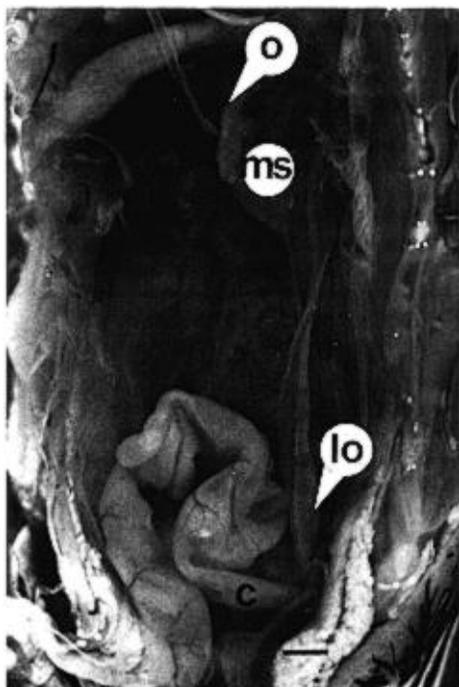


FIGURE 1. Reproductive organs of a partially eviscerated normal female Western Gull chick at hatching. A single left ovary (o) rests on the medial edge of the regressing mesonephros (ms). The left oviduct (lo) extends from the cloaca (c) to the border of the mesonephros. No right gonad is visible in this hatchling. ($\times 3$; scale bar 2 mm).

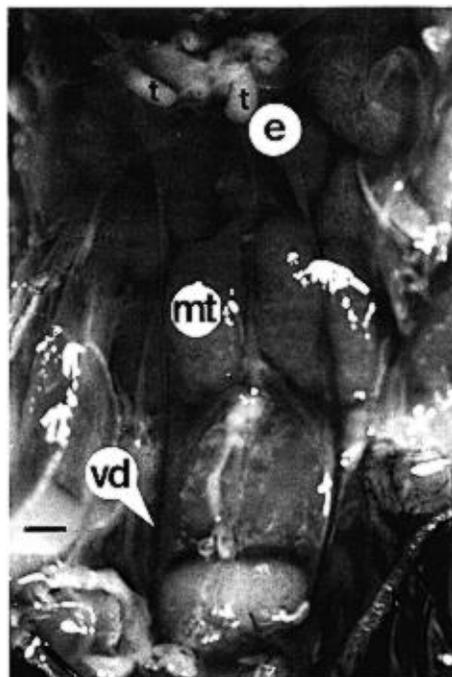


FIGURE 2. Reproductive organs of a control male California Gull chick at hatching. Left and right testes (t) are positioned at the cranial tip of the metanephros (mt). The mesonephros transforms into the epididymis (e) in males and the mesonephric ducts become the vasa deferentia (vd) which terminate at the cloaca. ($\times 3$; scale bar 2 mm).

shell thicknesses were measured by the Western Foundation for Vertebrate Zoology (WVZ), Los Angeles, CA. Shell thickness was determined for each egg and compiled for each clutch. Percent egg-shell thinning was determined from comparisons with 52 Glaucous-winged Gull egg shells in the WVZ collection which were gathered from the San Juan Islands, WA, prior to 1940.

RESULTS

EGG INJECTIONS AND EMBRYO DEVELOPMENT

Gross morphology of embryos at hatching

The anatomy of gull hatchlings and their response to estradiol or estrogenic compounds resembled those of chickens and Japanese Quail (Romanoff 1960, Lutz-Ostertag and David 1973), but gull embryos were more sensitive to the effects of estrogens, confirming the work of Boss (1943). Control female embryos had a prominent left ovary located at the cranial tip of the left kidney and a much reduced or absent right gonad (Fig. 1). Twenty of 21 control female embryos had a single left oviduct with a slightly thickened shell gland region adjacent to the cloaca. The left

oviduct is formed by differentiation of the left Mullerian duct, with the right Mullerian duct normally regressing during embryogenesis. One control female embryo had a vestigial right oviduct represented as a 5 mm tubule attached on the right side of the cloaca.

The reproductive anatomy of control male embryos is shown in Figure 2. Paired testes of approximately equal size were located at the cranial tip of both left and right kidney. The embryonic mesonephros differentiates into the epididymis, and the mesonephric ducts become the vasa deferentia that empty into the cloaca. Both left and right Mullerian ducts regress in normal male embryos by the time of hatching. Testis position and size varied little in control embryos. Left and right testes were 0.8–1.2 mm in length and most testes were symmetrical in size. Some embryos had right testis approximately 30% smaller than the left testis.

Estradiol injections

Estradiol at the lowest concentrations injected (0.5 ppm) caused extensive feminization of em-

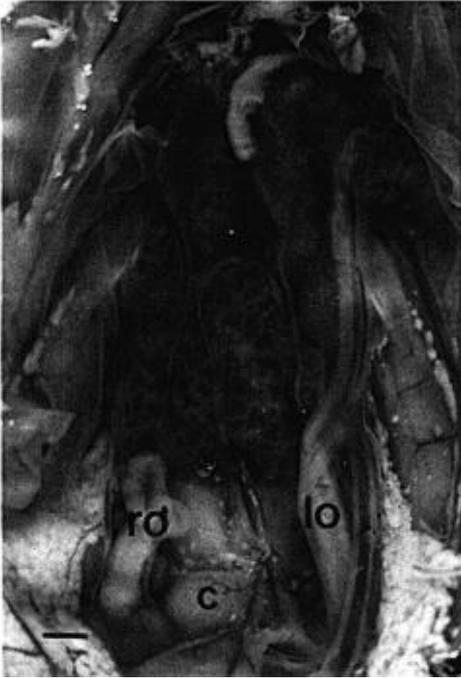


FIGURE 3. Female reproductive organs of a Western Gull chick injected with 0.5 ppm estradiol benzoate on day 1 of incubation. Both left oviduct (lo) and right oviduct (ro) extend from the cloaca (c). ($\times 3$; scale bar 2 mm).

bryos. The gross morphology of the reproductive tract exhibited both left and right oviducts in males and females (Fig. 3). The left oviduct was of normal female appearance, extending from the cloaca parallel to the left ureter and terminating in a funnel-shaped infundibulum adjacent to the left gonad. Right oviducts were less than half the length of left oviducts, frequently edematous, and sealed at the distal end. The gonads of both male and female embryos grossly resembled normal females, with 3–5 mm long left gonads and right gonads either not present or less than 1 mm in length. Males could not be separated from females by their gross morphology, and were identified from histological examination of sections of the gonad by the presence of seminiferous tubules in the left ovotestis (Fig. 11).

Organochlorine injections

The gross morphology of the reproductive tracts of embryos from eggs injected with organochlorine pollutants was intermediate between controls and estradiol injected eggs. O,p'-DDT (5 ppm and higher) and methoxychlor at high concentrations (20, 50, or 100 ppm) caused feminization of male embryos (Fig. 4) and per-

sistence of right oviducts in surviving female embryos (Table 1). Feminization of male embryos was identifiable from the presence of left, or left and right oviducts, with the left oviduct extending from a modified left ovotestis to the cloaca. Left oviducts in feminized males usually had a thickened shell gland region adjacent to the cloaca (Fig. 4). Right oviducts were short, 2–6 mm long, and frequently edematous. Left gonads of feminized males were elongated, frequently showing a raised cortical ridge down the ventral midline of the ovotestis. Right testes of feminized males were either of normal size or reduced in size by as much as 50%. Low doses of o,p'-DDT and methoxychlor resulted in reduced abnormalities or no gross alteration of testes. The mixture of p,p'-DDE plus p,p'-DDT (4:1) caused feminization of both male and female embryos at the high dose of 50 ppm (Fig. 5). Surviving embryos from eggs injected with p,p'-DDT or p,p'-DDE exhibited no gross abnormalities in either males or females.

Microanatomy of left gonads

In birds, males and female gonads develop from common primordia, but females differentiate in a sex-specific manner under the influence of estrogenic gonadal steroids. Without estrogenic hormonal influence, the primordial germ cells (PGC) migrate from the extra-embryonic membranes into the medullary tissue of the developing gonad which develops into a testis. Under the influence of estrogens, the PGC become localized in the cortex of the developing ovary. The histological anatomy of male and female gonads at hatching are distinct. Testes are circular or oval in cross section with a thin squamous epithelial cortex surrounding tortuous seminiferous tubules lined with prominently staining Sertoli cells and containing PGC that can be distinguished by their large nuclei and prominent nucleoli (Figs. 6, 7). PGC within seminiferous tubules remain in interphase and do not begin meiotic divisions into spermatogonia until the bird becomes an adult.

The left gonad of female embryos normally differentiates into a much flattened ovary under the influence of estrogen synthesized during differentiation (Fig. 8). Estrogens induce the PGC to become localized in a cortex of 100–150 μm thickness overlying medullary tissue consisting only of connective and vascular tissue. Primordial germ cells are prominent within the cortex and become organized in cords (Fig. 9). The morphology of the large primordial germ cells is distinctive with highly vacuolar cytoplasm and nuclei with condensed chromatin characteristic of meiotic prophase (Fig. 10). The large meiotic cells with condensed chromatin were used as a

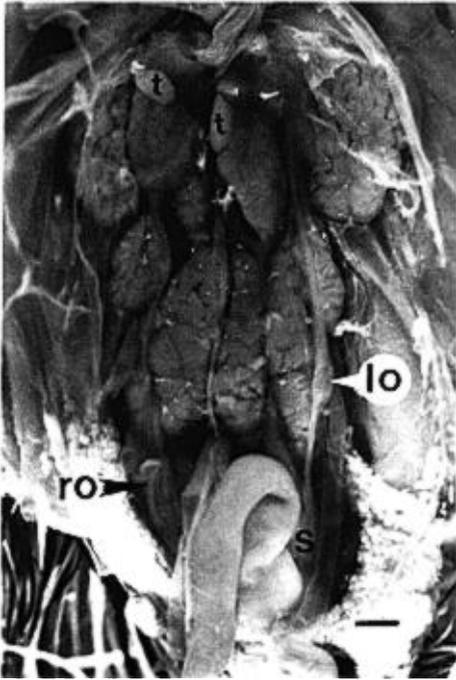


FIGURE 4. Reproductive tract of a male Western Gull hatchling injected with 5 ppm *o,p'*-DDT on day 1 of incubation. Testes (t) are of normal size and position. Feminization is indicated by presence of both short right oviduct (ro) and left oviduct (lo) with shell gland(s) at base of left oviduct ($\times 3$; scale bar 2 mm).



FIGURE 5. Reproductive tract of male Western Gull chick at hatching injected with a mixture of *p,p'*-DDT (10 ppm) and *p,p'*-DDE (40 ppm) on day 1 of incubation. The left gonad is enlarged and flattened into an ovotestis (ot). A left oviduct (lo) and an edematous short right oviduct (ro) are present. ($\times 3$; scale bar 2 mm).

marker of ovarian primordial germ cells. The PGC remain in prophase throughout juvenile and adult life and complete meiotic division just prior to ovulation.

The gonads of both male and female embryos injected with estradiol grossly resembled ovaries. Male gonads, however, contained seminiferous tubules with occasional primordial germ cells that enabled positive identification of genetic sex (Fig. 11). The ovaries of female embryos injected with estradiol appeared normal.

The extent of feminization of male gonads of embryos exposed to organochlorines varied between compounds and was dose-dependent. The most sensitive indicator of feminization was localization of PGC in a thickened cortex on the surface of the left testis (Figs. 12 and 14). Primordial germ cells in the cortex of feminized testes entered meiosis and the condensed chromatin and vacuolar cytoplasm produced a positive feminization marker. We classified testes as feminized only when PGC arrested in meiotic prophase were present in the cortex of the testis. The thickness of the cortex was variable between individuals and between the two species and was

not a reliable index of feminization. The lowest feminizing doses produced only scattered PGC in the cortex of the cranial tip of the testis, while increasing feminization resulted in clusters of PGC in a prominent ridge of cortical tissue extending the length of the elongated feminized left testis (Figs. 5, 12, 14). A second, but infrequently found, marker of feminization, described by Romanoff (1960), was the presence of seminiferous tubules which opened directly into the body cavity through the cortex of the testis (Fig. 13).

The most estrogenic DDT isomer was *o,p'*-DDT. Seven of 8 male embryos (87.5%) developed clusters of PGC in the cortex of the left testis at doses as low as 2 ppm (Table 1). Doses of 5 ppm and higher caused development of both left and right oviducts in addition to feminization of the cortex of the testes. The metabolite *p,p'*-DDE at high doses resulted in localization of primordial germ cells in the cortical tissue of half of the surviving male embryos. The mixture of *p,p'*-DDE and *o,p'*-DDT (4:1) at 50 ppm caused development of intersex testes. Methoxychlor induced the cortical localization of PGC

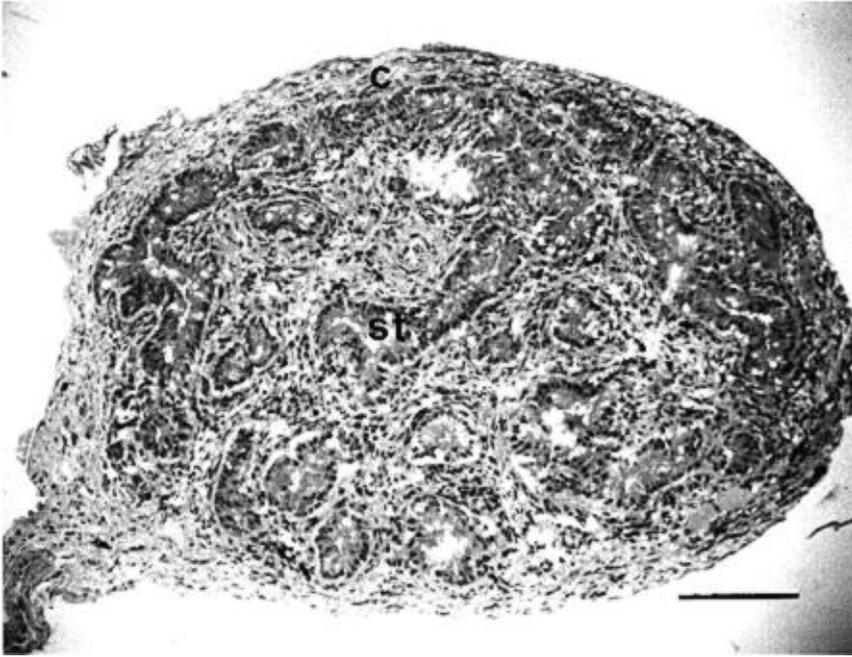


FIGURE 6. Left testis of a control male California Gull chick at hatching. The testis is oval in cross section with a thin fibrous cortex (c) surrounding a medulla containing many seminiferous tubules (st) cut obliquely and interspersed with interstitial connective tissue. (Paraffin embedded, hematoxylin and eosin stain (H&E); $\times 160$, scale bar $100 \mu\text{m}$.)

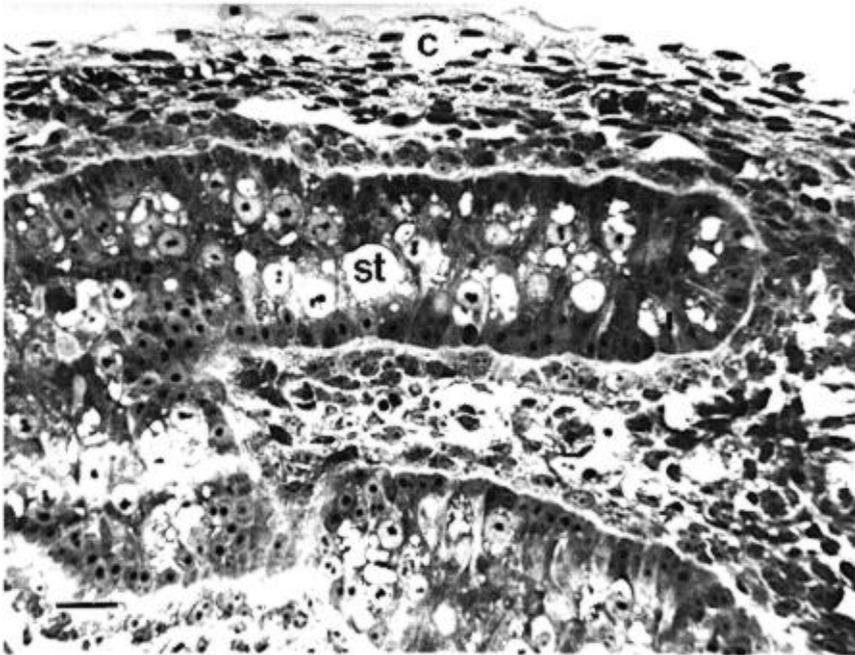


FIGURE 7. Left testis of a control male Western Gull chick at hatching. Seminiferous tubules (st) containing primordial germ cells with large interphase nuclei. Cortex (c) of testis is squamous epithelium. (Sorvall JB-4 embedded, basic fuschian and methylene blue stain (BF&MB); $\times 400$, scale bar $20 \mu\text{m}$.)

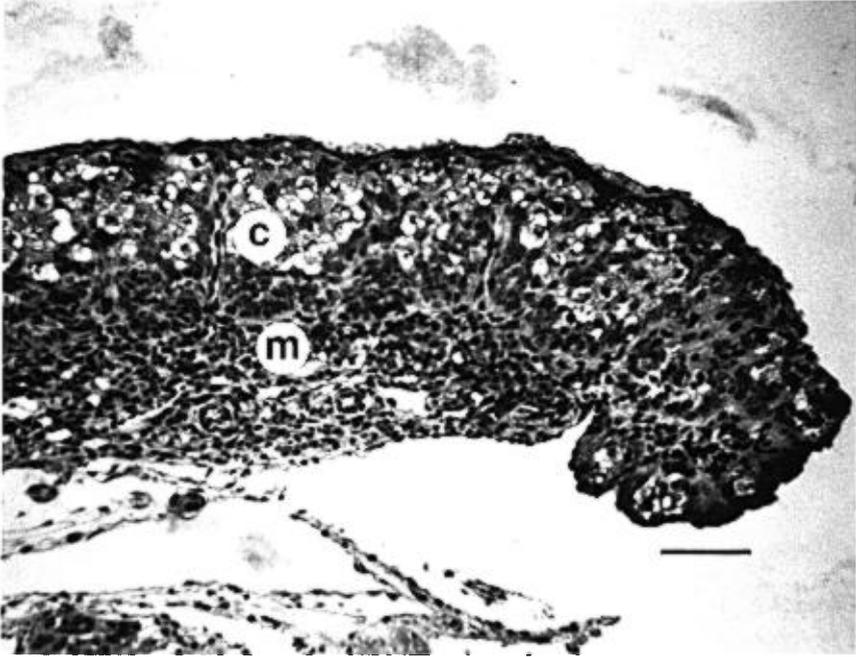


FIGURE 8. Left ovary of a control female California Gull chick at hatching. The ovary is flattened and differentiated into a cortex (c) containing primordial germ cells and a medullary region (m) containing vascular and connective tissue. (Paraffin embedded H&E; $\times 240$, scale bar $50 \mu\text{m}$.)

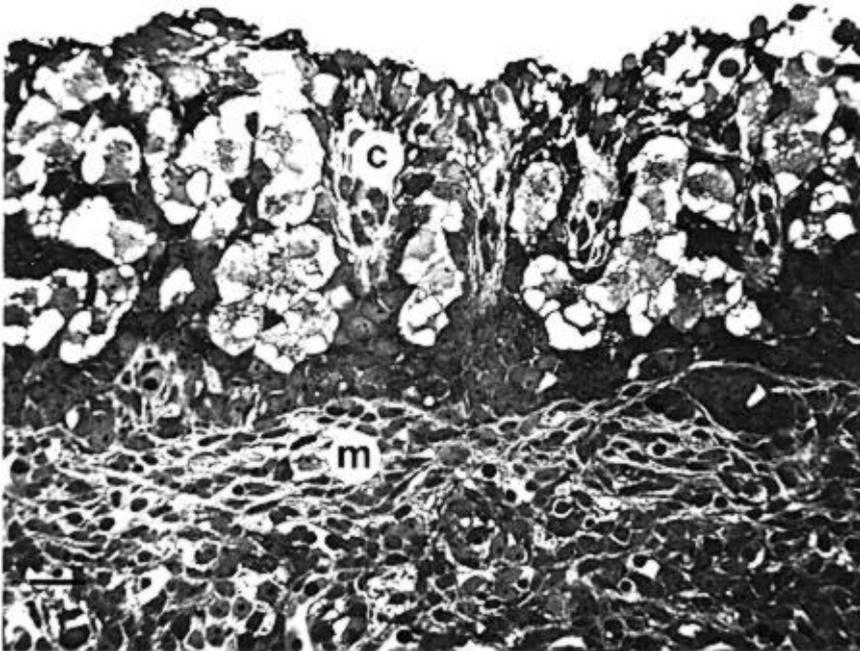


FIGURE 9. Left ovary of a control female Western Gull chick at hatching. Separation of cortex (c) and medulla (m) are distinct. Primordial germ cells are organized in cords within the cortex. (Sorvall JB-4, BF-MB; $\times 400$, scale bar $20 \mu\text{m}$.)

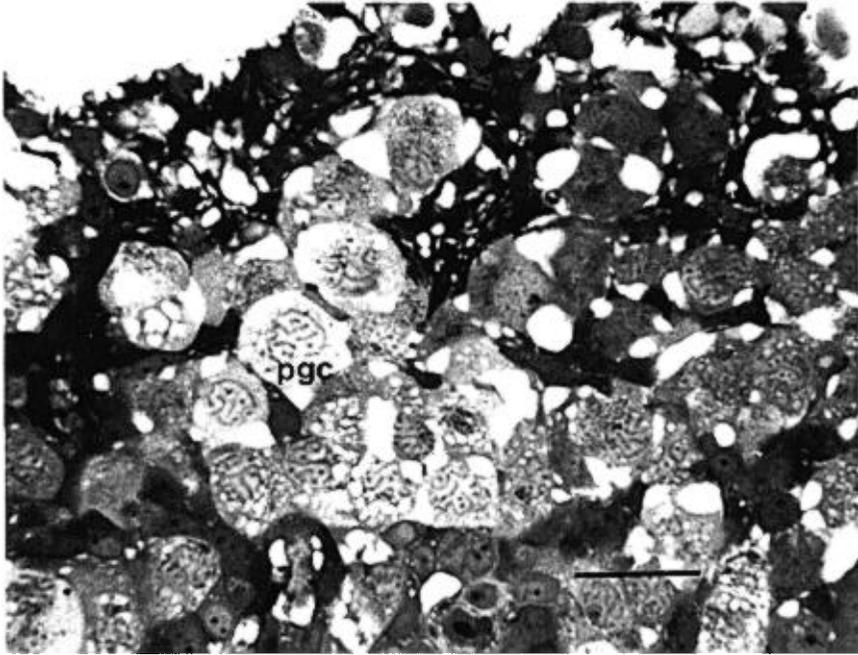


FIGURE 10. Cortex of left ovary of a control female Western Gull chick at hatching containing many primordial germ cells (pgc) with vacuolar cytoplasm and nuclei with thread-like condensed chromatin arrested in first meiotic prophase. (Sorvall JB-4, BF-MB; $\times 850$, scale bar $20 \mu\text{m}$.)



FIGURE 11. Left ovotestis of a male Western Gull chick at hatching injected with 2 ppm estradiol benzoate on day 1 of incubation. Testis has become flattened with a prominent cortex (c) containing primordial germ cells with vacuolar cytoplasm. Seminiferous tubules (st) lined with Sertoli cells positively identify the organ as a feminized testis. (Paraffin embedded, H&E; $\times 440$, scale bar $20 \mu\text{m}$.)

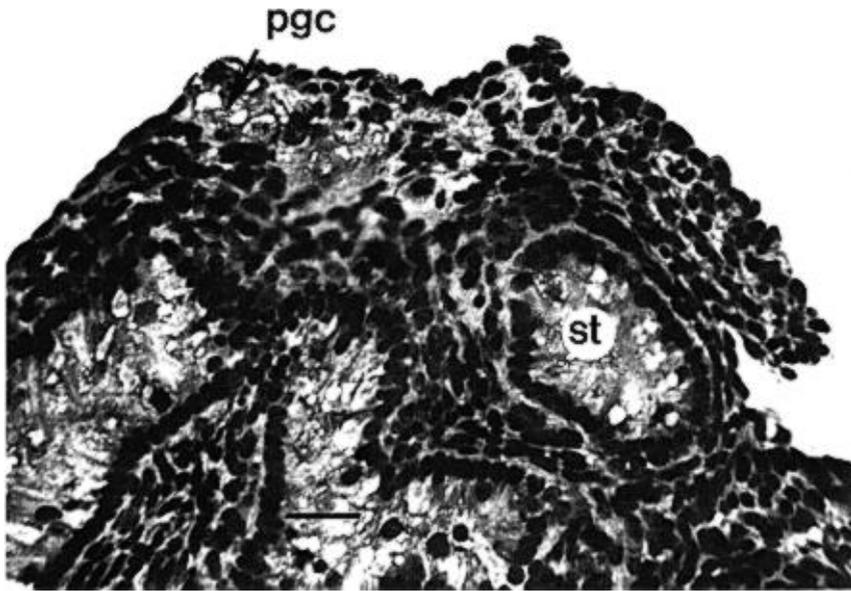


FIGURE 12. Left testis of a male Western Gull chick at hatching injected with 20 ppm o,p'-DDT on day 1 of incubation. Feminization is indicated by a thickened cortex containing clusters of primordial germ cells (pgc) arrested in first meiotic prophase. (Paraffin embedded, H&E; $\times 500$, scale bar 20 μm .)

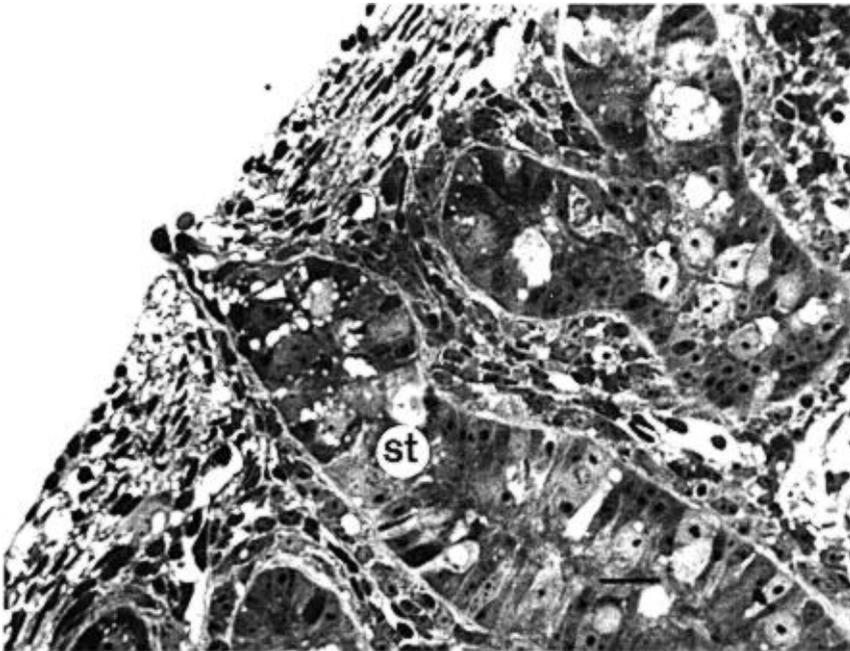


FIGURE 13. Left testis of a male Western Gull chick at hatching injected with 5 ppm o,p'-DDT on day 1 of incubation. A seminiferous tubule (st) opens through the cortex directly into the body cavity. (Sorvall JB-4 embedded, BF-MB; $\times 440$, scale bar 20 μm .)

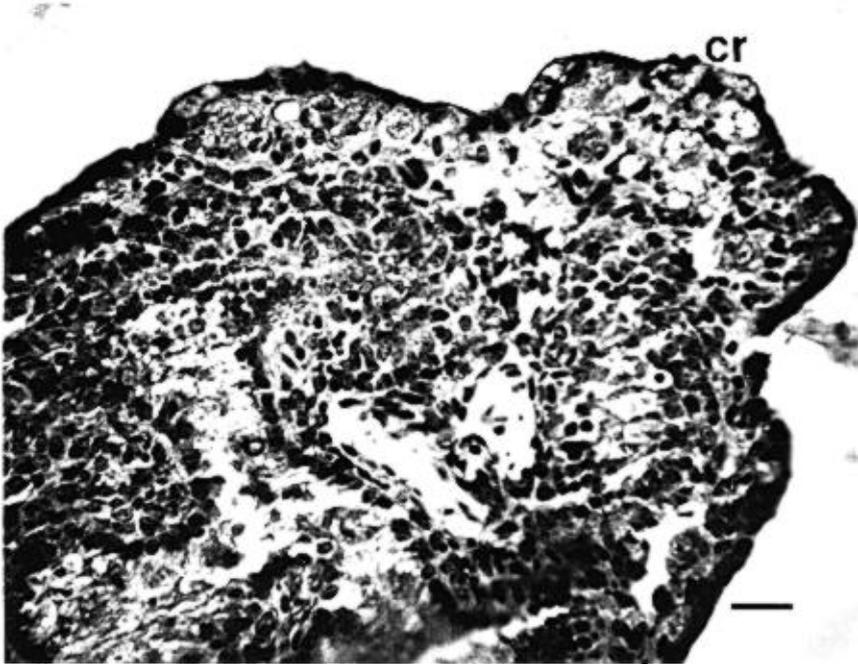


FIGURE 14. Left testis of a Western Gull chick at hatching injected with 100 ppm methoxychlor on day 1 of incubation. Feminization is indicated by thickening of the cortex into a cortical ridge (cr) containing many primordial germ cells arrested in first meiotic prophase. (Paraffin embedded, H&E; $\times 420$, scale bar $20\mu\text{m}$.)

at 2 ppm, and 4 of 5 male embryos surviving doses of 50 and 100 ppm developed both ootestes and left and right oviducts (Fig. 14). p,p'-DDT did not result in feminization of any male embryos or persistence of right oviducts in any female embryos.

SURVEY OF GLAUCOUS-WINGED GULLS IN PUGET SOUND

Reproductive anatomy

The number of gulls collected in each area and reproductive tract comparisons are presented in Table 2. Female gulls trapped on nests with 3 eggs all had ovaries with 3 postovulatory follicle stalks and enlarged left oviducts that were beginning to regress. The extent of oviduct regression was correlated with the age of embryos observed when eggs were blown. Birds with the most regressed oviducts were incubating eggs with the largest embryos.

Twenty-one of 31 female gulls had right oviducts, 13 with right oviducts greater than 10 mm in length. The incidence and size of right oviducts observed in birds in this study varied with colony location. Most birds from Goose Island (5 of 6, 83%) and Seattle (5 of 6, 83%) had completely regressed or small right oviducts (less than 5 mm); this is the expected normal development. All birds

from Tacoma (6 of 6) had right oviducts larger than 10 mm. Birds from Smith Island and Shelton were intermediate in both frequency and size of right oviducts.

Egg-shell thickness

Average egg-shell thicknesses of clutches combined by colony are given in Table 3. Egg-shell thicknesses from the two relatively unpolluted reference sites (Smith Island and Goose Island) were not significantly different from eggs collected in the San Juan Islands prior to the use of DDT (Table 3). Eggs from the 3 target sites of Seattle, Tacoma, and Shelton had average egg-shell thinning of 10%, 9%, and 8%, respectively. This is a marked amount of thinning for a gull species, and is comparable to thinning caused by high levels of DDT in Lake Michigan in the mid-1960s (Keith, 1966; Hickey and Anderson, 1968).

Supernormal clutches of eggs in Puget Sound

The mean clutch size in colonies of Glaucous-winged Gulls ranged from 2.21–2.86 eggs per nest (Table 4) and clutch size frequencies were similar to those recorded by Vermeer (1963) for Glaucous-winged Gulls breeding on Mandarte Island, B.C., in 1961. Three clutches of 4 eggs

TABLE 1
REPRODUCTIVE ANATOMY OF GULL EMBRYOS
EXPOSED TO ORGANOCHLORINES OR ESTRADIOL BY
INJECTION IN OVO

Treatment group	Dose	Normal males	Feminized males	Normal females	Abnormal females
Control		10	0	20	1
o,p'-DDT					
Low	2, 5	1	5**	2	2
High	20, 50, 100	0	2*	0	5***
p,p'-DDT					
Low	2	2	0	2	0
High	20, 100	2	0	3	0
p,p'-DDE					
Low	2	1	0	1	0
High	20, 100	3	3*	2	0
p,p'-DDT and p,p'-DDE (4:1)					
Low	5	1	1	2	1
High	50	0	2*	2	2
Methoxychlor					
Low	2, 5	0	3**	1	1
High	20, 50, 100	0	5***	1	6***
Estradiol					
Low	0.5, 2	0	5***	1	3**
High	5, 20	0	5***	1	0

* Different from controls $P < 0.05$.

** Different from controls $P < 0.01$.

*** Different from controls $P < 0.001$.

Significance determined using Fisher's Exact Test.

Data from Fry and Toone 1981.

were observed from a total of 1164 clutches. Two of the SNC were in Seattle (2.1% of clutches) and 1 at Goose Island (0.16% of clutches).

DISCUSSION

ESTROGENIC COMPOUNDS AND EMBRYONIC DEVELOPMENT

We have shown that estrogenic organochlorines are teratogenic and cause abnormal development of the reproductive tract in gulls. Exposure of domestic chicken (Wilson and Glick 1970), Japanese Quail (Adkins 1975, 1979), or Zebra Finch (Gurney and Konishi 1980) embryos to estrogens causes both feminization of the reproductive tract and "demasculinization" of the developing brain. The latter results in permanent suppression of sexual behavior in male birds when they become adults. Gulls are particularly susceptible to effects of estrogenic teratogens as shown by this study and that of Boss (1943) and the data support the conclusion that gulls are 10–50 times more sensitive than chickens, Japanese quail, or finches. It is not known whether the levels of estrogenic substances that

TABLE 2
REPRODUCTIVE TRACT COMPARISONS OF
GLAUCOUS-WINGED GULLS, WESTERN WASHINGTON,
1984¹

Location	Number examined	Right oviducts present		Right oviducts >10 mm	
		N	%	N	%
Goose Island	6	1	17	1	17
Smith Island	6	5	83	3	50
Seattle	6	3	50	1	17
Shelton	7	6	86	2	29
Tacoma	6	6	100	6	100

¹ Data from Calambokidis *et al.* 1985.

cause gonadal changes in male gulls would also cause behavioral changes, but such changes occur in tandem in all other bird species which have been studied.

The estrogenic effects of DDT on developing chickens and quail parallel the results of this study. The greater sensitivity of gulls compared to chickens to the morphological feminizing effects of o,p'-DDT and methoxychlor has been confirmed in this study. Additionally, mixtures of p,p'-DDT plus p,p'-DDE also caused feminization, although requiring higher doses.

Exposure of embryos to estrogenic substances does not lead to complete sex reversal, but results in modification of the reproductive tract of both males and females. The breeding behavior of exposed females, however, is not altered by exogenous estrogens (see review by Ottinger *et al.* 1984). Female gull embryos in this study exhibited persistence of right oviducts with exposure to exogenous estrogens, and these changes may be associated with eggshell thinning and abnormal eggs when exposed birds become adults. Greenwood and Blyth (1938) found that chick-

TABLE 3
EGGSHELL THICKNESS (WITH MEMBRANE) OF WHOLE
EGGS OF GLAUCOUS-WINGED GULLS, WESTERN
WASHINGTON, 1984¹

Location	Number eggs measured	Mean thickness mm (SD)	Per cent change from pre-1940 ²
Pre-1940, San Juan Islands	52	0.395 (.025)	—
Goose Island	33	0.388 (.029)	-2
Smith Island	16	0.384 (.035)	-3
Seattle	13	0.354 (.032)	-10**
Shelton	29	0.362 (.025)	-8**
Tacoma	20	0.360 (.028)	-9**

¹ Data from Calambokidis *et al.* 1985.

² Measurements of eggs from the San Juan Islands.

** Significantly different from pre-1940 eggs from the San Juan Islands ($P < .001$).

TABLE 4
CLUTCH-SIZE DISTRIBUTION AND MEAN CLUTCH SIZE OBSERVED FOR NESTS OF GLAUCOUS-WINGED GULL
COLONIES, WASHINGTON, 1984¹

Location		Clutch size				Total clutches counted	Mean clutch size (\pm SD)	Survey dates
		1	2	3	4			
Goose Island	n =	133	219	259	1	612	2.21 (0.78)	8 June
	% =	22	36	42	<1			
Smith Island	n =	14	37	59	0	110	2.41 (0.71)	14 June
	% =	13	34	54	0			
Olympia	n =	0	3	27	0	30	2.90 (0.31)	May/June
	% =	0	10	90	0			
Seattle	n =	3	22	95	2	122	2.79 (0.50)	June/July
	% =	2	18	78	2			
Shelton	n =	8	29	83	0	120	2.63 (0.61)	18 June
	% =	7	24	69	0			
Tacoma	n =	4	21	175	0	200	2.86 (0.41)	June/July
	% =	2	11	88	0			

¹ Data from Calambokidis et al. 1985.

ens raised from estrone-injected eggs had abnormal oviducts and laid thin-shelled or abnormal eggs.

REPRODUCTIVE ABNORMALITIES AND CHEMICAL CONTAMINANTS IN PUGET SOUND

Both the persistence of right oviducts and egg-shell thinning in Glaucous-winged Gulls in Puget Sound may be consequences of pollutant induced developmental abnormalities. Localized areas of Puget Sound have historical contamination, principally of PCB and polyaromatic hydrocarbons, but not extensive amounts of DDT (Riley et al. 1983). Inorganic and organic pollutants have been discharged into harbors of Puget Sound for many years (Malins et al. 1980, Riley et al. 1983). The major classes of contaminants in central and southern Puget Sound consisted of moderate concentrations of heavy metals (copper [Cu], zinc [Zn], arsenic [As], silver [Ag], antimony [Sb], mercury [Hg], and lead [Pb]), polychlorinated biphenyls (PCB), polynuclear aromatic hydrocarbons (PAH), and low to moderate concentrations of chlorinated butadienes. The highest concentrations of all pollutants were located in Commencement Bay adjacent to Tacoma and in Elliot Bay adjacent to Seattle. DDE was detected in most samples of fish and birds, but no DDT or DDD was detected in any samples. Riley et al. (1983) monitored contaminant levels in adult Great Blue Herons and in nestling Glaucous-winged Gulls, finding that Great Blue Herons from the Seattle and Tacoma areas had liver residues of PCB's of 1.0–5.1 ppm and concentrations in the fat of 14.6–80.4 ppm. Great Blue Herons from Sequim Bay on the straits of Juan de Fuca had much lower concentrations of PCB's

(0.75 ppm liver, 5.5 ppm fat). Nestling gulls had low concentrations of PCB's, as would be expected from young birds, with levels in birds from Tacoma ranging from 0.81–1.1 ppm in liver and in Seattle ranging from 0.12–0.28 ppm in liver. Residues in gull nestlings from Protection Island were correspondingly lower (0.2–0.8 ppm). Small amounts of DDE were detected, but not quantified, in each of the samples of Great Blue Herons and gull nestlings.

The birds from urban areas of Puget Sound have had abnormally high concentrations of PCB relative to documented concentrations in marine birds from other areas of the world. The extent of organochlorine pollution in Puget Sound, as measured from tissue residues of herons and gulls in 1982, is low compared to organochlorine pollutants in parts of the Great Lakes and in southern California in the 1970s. Analysis of sediments in Puget Sound, however, indicates that historically higher levels of pollution existed prior to 1980 and that pollutants may have contributed to the death of marine birds through direct poisoning or through sublethal effects on birds at times of environmental stress (Riley et al. 1983).

The correlations between areas of moderate contamination, egg-shell thinning, and incidence of right oviducts is striking. In 1979 G. Eddy (pers. comm.) made a survey of the nests on warehouse roofs in Smith Cove, Seattle, and found 14 of 67 nests (21%) held clutches of 4 eggs. He repeated the survey in 1981 observing 147 nests, but none held 4 eggs. This extremely high incidence of SNC in 1979 remains unexplained. From the studies of Hunt et al. (1980), Kovacs and Ryder (1981, 1983), Fox and Boers-

ma (1983), and Conover (1984b), the presence of SNC appears to accurately predict the occurrence of female-female pairing, but may underestimate the actual number of female-female pairs within a colony (Conover and Aylor 1985).

The incidence of SNC in Glaucous-winged Gulls in unpolluted areas is rare. Conover (1984a) compiled data from the literature and reported an occurrence of 0.1% for more than 5000 clutches. Vermeer (1963) working on Mandarte Island, B.C., observed zero SNC of 479 clutches in 1961. Galusha (pers. comm.) surveyed Glaucous-winged Gull nests on Protection Island in the Straits of Juan de Fuca and observed 1 SNC of 3909 clutches in 1980, 0 of 4057 in 1982, and 1 of 4734 in 1984.

The episode of extremely high density of supernormal clutches in 1979 and the continuing moderate number of supernormal clutches in Seattle is consistent with the distribution of pollutants. The actual causes of persistent right oviducts in females and egg-shell thinning in Puget Sound is unknown, however, as only trace amounts of DDE were detected by Riley et al. (1983) in surveys conducted in 1981 and 1982.

ORGANOCHLORINE POLLUTANTS AND GULL REPRODUCTION IN OTHER AREAS

The parallel distribution of organochlorine pollutants and female-female pairing correlates well with data from the Great Lakes and southern California, both in historical occurrence of SNC and geographical location. Incidence of SNC in Herring and Western gulls increased markedly subsequent to the introduction of DDT and other organochlorine insecticides after World War II (Conover 1984a, Conover and Hunt 1984b).

Polygyny and female-female associations of Herring Gulls occurred in northern Lake Michigan (Shugart 1980) in areas contaminated with high levels of DDT (Keith 1966, Strachan and Edwards 1984). The distribution of other Herring Gull colonies having polygyny and female-female pairing correlates geographically with the distribution of organochlorine pollution in Lake Ontario and the other Great Lakes (Gilman et al. 1977, Mineau et al. 1984, Gilbertson and Fox 1977, Fox et al. 1978, Fox and Boersma 1983).

Gulls and other seabirds in southern California were highly contaminated with DDT and DDE residues throughout the 1960s and early 1970s (Risebrough et al. 1967, Gress et al. 1973, Anderson et al. 1975). Southern California DDT pollution was particularly damaging because the estrogenic isomer *o,p'*-DDT was present in the food chain (MacGregor 1974) and was detected in Brown Pelican eggs (Lamont et al. 1970). MacGregor (1974) reported that *o,p'*-residues from myctophid fish averaged 22.3% of total-

DDT throughout the period from 1949 to 1973 as DDT accumulated in southern California offshore waters. Total-DDT residues in fish averaged more than 4 ppm from 1966 through 1973. Total-DDT residues in Brown Pelican eggs in 1969 (Lamont et al. 1970) averaged about 80 ppm (wet-weight basis) with *o,p*-DDT and *o,p'*-DDD averaging 7.9% of the *p,p'*-isomers, a very significant amount of the estrogenic isomer. Brown Pelicans and Double-crested Cormorants showed extensive egg-shell thinning and reproductive failure due to organochlorine contaminants, but because gulls are moderately insensitive to egg-shell thinning by DDT (Hickey and Anderson 1968), continued breeding was possible and embryos were exposed to high levels of organochlorine contaminants. The overall effect of the organochlorine pollution in southern California was to cause the complete failure of reproduction in Brown Pelicans and cormorants and to reduce the productivity of the gull colonies.

The fitness and ultimate survival of gulls hatched from contaminated eggs in the wild is unknown. The demography of the gull colonies in southern California described by Hunt et al. (1980) indicates only a marked excess of breeding females. Without direct information from the field, it cannot be known whether male gulls hatched from contaminated eggs had decreased survival compared to females or whether feminization resulted in suppression of sexual behavior and self-exclusion from the breeding colony.

REDUCED NUMBERS OF MALE GULLS IN BREEDING POPULATIONS

Sex-ratio skew in breeding populations of gulls in polluted areas has been postulated to be the result of selective mortality of males (Hunt et al. 1980, Shugart 1980, Nisbet and Drury 1983), although no evidence that male gulls are more sensitive to pollutants has been demonstrated. If male gulls, as adults, are more sensitive to organochlorine pollutants, a functional alteration in the sex ratio of the population could be achieved by differential survival of females in addition to the exclusion of developmentally feminized males from the breeding population. These possibilities are, of course, not mutually exclusive as both adults and embryos would typically be exposed to environmental contaminants and the reduced number of males in breeding colonies could reflect effects on embryos as well as on juveniles and adult males.

The presence of female-female pairs in a breeding colony suggests a skew in the operational sex ratio of the colony, but there are limited data on actual sex ratios in most colonies.

It has been suggested that sex ratios skewed to females in new or rapidly expanding colonies results because of a greater dispersion of females from natal colonies (Greenwood 1980, Fox and Boersma 1983, Fetterolf et al. 1984), although confirmation of the sex ratio has not been made in any of the Ring-billed Gull colonies studied. In the Western Gull colony on Santa Barbara Island which had a high incidence of female-female pairing and SNC, Hunt et al. (1980) determined the sex ratios of nesting pairs and non-breeding birds attending "bachelor clubs" and found a marked excess of females (19 females per male). Mills (1973), Burger and Gochfeld (1981), and Spear et al. (this symposium) have also documented surpluses of young breeding-age females in clubs of non-breeders, even in colonies that do not have supernormal clutches.

NEST SITE AVAILABILITY

As sex-ratio skew occurs in some colonies without female-female pairing, the additional factor of reduced competition for available nest sites appears to be of importance to allow the formation of female-female pairs and nesting. In areas of high breeding density, female gulls do not compete as successfully for territories as breeding males, Kovacs and Ryder (1981, 1983) reported that female-female pairs of Ring-billed Gulls breeding in high density colonies often have smaller territories than normal pairs and have greater difficulty in maintaining sites from year to year. Fetterolf et al. (1984) observed nest-site distribution within colonies and found female-female pairs in many colonies tended to be clustered, often occupying territories which became free during the season, such as spaces between large territories, or in areas of beach that became exposed after nesting had commenced.

Hand (1980) postulated that female-female pairs could establish territories even in relatively dense colonies as long as all breeding males also had adequate territories. In colonies with declining populations such as Santa Barbara Island, or in colonies with an artificially reduced number of males such as the colonies experimentally manipulated by Conover and Hunt (1984a), the availability of nest-sites is increased and affords female-female pairs a greater probability to establish breeding territories.

Even when a slight excess of breeding-age females exists, competition for territories may be responsible for the absence of female-female pairs in established, stable colonies of gulls. Female-female pairing and supernormal clutches are extremely rare in Atlantic maritime colonies (Nisbet and Drury 1984). A survey by Burger and Gochfeld (1981) in New England and New York, however, indicated that the composition of adult

birds in established Herring Gull colonies averaged 52% females. "Bachelor clubs" of unpaired adults had a slightly greater sex-ratio skew of approximately 61% females. Similar data have been presented by Spear et al. (this symposium), who demonstrated a skewed sex ratio of non-breeding adult gulls on Southeast Farallon Island, CA, a very densely populated Western Gull colony with no occurrence of supernormal clutches. The sex ratio of the small number of non-breeders (about 700 out of a total population of 25,000) was 2.04 females per male. It is possible that given available nesting territory, some of the excess young females would form pairs and attempt to nest.

Why do surplus female gulls form female-female pairs? Both male and female gulls defend the breeding territories, although males are most vigorous in this activity (Pierotti 1981, Hand 1986). Normal female gulls have high circulating levels of androgens (Wingfield et al. 1980) that correlate with aggressiveness and territoriality. This is not unusual, as females of most species that defend territories have levels of circulating androgens comparable to levels in males of the same species (Wingfield 1983). Females that enter into female-female pairs appear to be normal behaviorally as they have female behavior and do not adopt male behavior patterns in courtship or defense of territories (Hand 1981, Hunt et al. 1984). In colonies with a reduced number of males, female-female pairing by gulls appears to occur as a matter of course. It is indeed remarkable that at least some female gulls would persist in expending much energy when only rarely are female-female pairs successful in raising chicks (Kovacs and Ryder 1983, Hunt and Hunt 1977).

SUMMARY

Female-female pairing occurs in two demographic situations: (1) newly formed or rapidly expanding colonies of Ring-billed and California gulls nesting in the Great Lakes region and in the Pacific Northwest; and (2) populations of Herring, Western and Glaucous-winged gulls breeding in areas polluted with organochlorines. Influx into expanding colonies appears to occur with a sex ratio skewed to females because of increased dispersal of young breeding-age females from their natal sites. Sex-ratio skew of colonies in polluted areas may occur as a result of decreased fitness of male gulls hatched from contaminated eggs or decreased fitness of juvenile and adult male gulls exposed to organochlorine pollutants.

A skewed sex ratio alone is not sufficient to result in formation of female-female pairs. Reduced competition for available nest sites appears to be necessary if females are to defend territories successfully. Colonies having both of

the required conditions (skewed sex ratio and available nest sites) are expanding colonies with excess nest sites and colonies with reduced populations in polluted areas where nest sites have become available due to a decrease in the male breeding population. Female-female pairing and reduced reproductive success of gulls in southern California and the Great Lakes is a striking example of pollutant effects on entire populations of birds.

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