

EGGSHELL QUALITY: ITS ECOLOGICAL AND PHYSIOLOGICAL SIGNIFICANCE IN A DDE-CONTAMINATED COMMON TERN POPULATION

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Recent ornithological literature contains many references implicating organochlorine environmental pollutants, particularly DDE, in eggshell thinning and decreased reproductive success in bird- and fish-eating birds (see reviews by Cooke 1973, Jefferies 1973). The avian eggshell serves as a rigid container, a microbial barrier, the source of skeletal calcium, and the principal resistance to gaseous exchange for the developing embryo. All of these functions are presumably important to the successful hatching and survival of young birds.

In this paper I discuss the first field study in which the fate of individual eggs, of *known shell quality*, was followed in a single DDE-contaminated nesting population, and in which the chemical, ultrastructural, and respiratory characteristics of shells of eggs which failed to hatch were examined.

METHODS

Between 20 May and 20 August 1972, I made 44 visits to a colony of Common Terns (*Sterna hirundo*) nesting on a small island in Buffalo Lake, Alberta, Canada (52° 20' N, 112° 50' W). Each fresh egg was marked with the nest number and its sequence within the clutch and weighed to the nearest 0.01 g. I searched the colony for new nests on each visit, and recorded the contents of each nest at 48-hour intervals, weather permitting. All eggs were examined for cracks, dents, or evidence of pipping each time the nest was checked. Eggs which were cold when examined were placed on their large pole, and if they remained in that position on the next visit, they were regarded as abandoned.

Eggshell quality.—I measured the shell quality of each egg with a specially built portable beta-backscatter (BBS) gauge equipped with a 10 μC^{106} Ruthenium beta source (Fox et al. 1975, James and Retzer 1967, Voisey et al. 1969). The egg was suspended over the weak beta source and those beta particles which were reflected (backscattered) by the shell were detected by a Geiger-Mueller tube connected to a timer and counter. Backscattering is a simultaneous response to 3 distinct properties of the target: the mass of the constituents, their depth distribution, and crystalline structure (Nicolet et al. 1972). Hence, BBS is a simultaneous measure of thickness, density, and ultrastructure. It correlates highly ($r_s = 0.96$, $P < 0.01$) with the thickness index of Common Tern eggshells. The procedure adopted measured 3 well-separated areas on the waist of the egg, each of which was about 1 cm in diameter, and should be representative of the shell as a whole. Exposure of eggs to beta radiation, under these conditions of measurement, had no effect on hatchability.

Evaporative water loss of 50 randomly selected eggs was determined by weighing the egg on 2 or more visits. The surface area of the egg was calculated as $4.835 \times \text{fresh egg weight}^{0.602}$ (Paganelli et al. 1974) and used to calculate the evaporative water loss in mg/cm^2 per day. Only 46 of these eggs were incubated to term.

Eggs which failed to hatch, were damaged, or were abandoned, as well as samples of fresh eggs representing the range of BBS values encountered, were collected. Their contents were blown into labeled, pre-weighed hexane-rinsed glass jars with aluminum foil-lined lids and stored at 15°C. The inside of the shell was rinsed repeatedly with distilled water and then allowed to drain and air dry for 4 months at room temperature.

Lengths and breadths of air-dried shells were measured to the nearest 0.1 mm with a dial caliper. Their weight was determined to the nearest 0.001 g, or in the case of eggs in museum collections, 0.01 g. These measurements were used to calculate the thickness index (Ratcliffe 1967). Thickness indices were calculated for 87 Common Tern eggs from the collections of the National Museum of Natural Sciences (Ottawa), the Royal Ontario Museum (Toronto), and the Museum of Vertebrate Zoology (Berkeley). These eggs were collected from 9 colonies in Alberta, Saskatchewan, and Manitoba in 8 years spanning the period of 1894 to 1940 and should represent the geographic, temporal, and intra-clutch variability which existed in shells of this species on the Canadian Prairies prior to the introduction of DDT.

Samples (ca 1 cm²) were cut from the waist of 31 shells using an abrasive disc. The average thickness of these samples with attached membranes was measured to 0.005 mm with a modified dial micrometer. The samples were weighed to 0.001 g, boiled in 2.5 N sodium hydroxide until the membranes were released, rinsed in distilled water, and allowed to digest in 10.0 ml of 0.1 N hydrochloric acid. Calcium content of the digest was determined by flame emission spectrophotometry, the magnesium content by atomic absorption spectrophotometry, and the phosphorus content colorimetrically by the method of Harwood et al. (1968). Calibration was based on dilutions of an aqueous standard.

Small fragments (ca 3 mm²) were removed from the waist of selected shells for scanning electron microscopy. Three fragments were mounted on a single stub. One, untreated, was mounted face up. Another, treated with boiling 2.5 N sodium hydroxide to remove the membranes, was mounted face down. Pieces of untreated shell which had been cleanly broken from the egg were mounted in a perpendicular position. Thus it was possible to view the external surface, the internal surface with membranes removed, and the structural elements in sagittal section by examining 1 stub. The stubs with mounted shell fragments were coated with gold and examined using a Cambridge Stereoscan 4 scanning electron microscope. Photographs of representative and selected portions of these fragments were taken at standard magnifications. Contact prints of the resulting 58 cm² images were used for study and quantification. The total number of pore openings and the total number of mammillary bodies present in a representative field of the internal surface were recorded. All other observations were of a qualitative nature. There was much variation in the distribution of ultrastructural elements within the total surface area. However, by selecting the sample from the waist, which is the most uniform region of the shell, and by quantifying a representative rather than an exceptional field, I hoped that bias would be reduced and statistical comparison would be valid.

Organochlorine analysis.—The frozen egg contents were thawed and weighed to the nearest 0.01 g. The weight of the homogenate used in the clean-up procedure was corrected to the equivalent of 5.0 g of fresh egg contents by the following formula: 5.0 g × weight of total homogenate ÷ (weight of fresh egg ÷ weight of air-dried shell). Food items were homogenized in their entirety. The homogenate was transferred to a mortar containing 50 g of florisil and ground to a free-flowing powder. This was added to a glass chromatographic column containing 50 g of florisil prewashed with methylene chloride:petroleum ether (1:1, V/V). The mortar and pestle were washed twice with 100 ml of petroleum ether:methylene chloride (4:1, V/V) and these washes added to the column. The eluate

was collected in a 1-liter round bottom flask and flash evaporated to dryness. The residue was then dissolved in 10 ml of n-hexane. A Varian Aerograph 600 D gas-liquid chromatograph with a 250 μ c tritium source electron capture detector and a 1.6 m glass column packed with a matrix of 10% DC-200 and 15% QF1 on 80/90 mesh Anakrom ABS was used for the separation and quantification of the compounds. The carrier gas was purified nitrogen at a flow rate of 70–85 ml/min. The oven temperature was 175° to 185°C. Only p', p'DDE was quantified as it was the only organochlorine present in appreciable quantities. Sensitivity of detection of DDE was 0.05 ppm, and reproducibility of recoveries was $\pm 0.25\%$.

Behavior.—One egg in each of 80 nests was displaced approximately 10 cm from the nest scrape, early and late in incubation, during special visits to the colony. The observer returned to the colony 30 min later and recorded whether or not the eggs had been returned to the nest scrape in the test period. Baerends (1969) has recommended egg displacement as a method of measuring the intensity of incubation instinct in larvae.

All chicks were banded with a Fish and Wildlife Service band when first observed, and every attempt was made to ascertain the identity of the egg from which they hatched. The identity and condition of all chicks encountered was recorded on each visit. Depth perception and motivation of 55 chicks were tested on a visual cliff at 3 to 8 days post hatching. The visual cliff consisted of a 40 cm \times 35 cm clear plexiglass platform immediately overlaying a sliding plywood "cliff" 30 cm deep, which was painted in a 2.5 cm² black checkerboard pattern, surrounded by a 15 cm high wooden fence. The testing procedure was to locate the "cliff edge" in the middle of the fenced field, and to place the chick on the plexiglass so it was looking over the "cliff edge." Chicks were allowed 3 min to choose the shallow or deep side by stepping backward or forward, respectively. Care was taken to ensure that the chick actually looked down at the plexiglass floor and "cliff edge" at least once during the test period. If the chick did make a choice, the procedure was repeated by sliding the "cliff" into a new position to correspond with the chick's new position, and the test repeated. Chicks which did not react in 3 min were recorded as "no response." If for any reason the response was questionable, the individual was retested later during the same visit or on the next visit. Chicks which were tested on 2 or more occasions were consistent in their response. An animal's response to the lack of visual support is unlearned (Walk and Gibson 1961). In the gulls, *Larus argentatus* and *L. atricilla*, a negative edge response is detectable in chicks less than 24 hours old (Emlen 1963, Hailman 1968).

Statistical analysis.—Non-parametric statistics were applied whenever possible as they are sensitive to differences in small samples and make no assumptions as to the nature of the population distribution or its variance (Siegel 1956).

NESTING SUCCESS

The Common Terns laid 473 eggs in 226 nesting attempts, resulting in a mean clutch size of 2.1 eggs. Of these eggs, 288 (61%) hatched. At least 133 of these 288 chicks (46%) survived 14 days. Eighty-three % of chick mortality occurred within the first 14 days, hence the figures of 0.28 14-day-old chicks per egg laid (0.59 per nesting attempt) closely approximates nesting success. Nisbet (pers. comm.) estimates that a minimal production of 1.1 fledged chicks/pair per year is necessary to maintain stability in the eastern seaboard population.

Langham (1968) and Nisbet (pers. comm.) have made intensive studies

TABLE 1
FATE OF EGGS FROM 3 POPULATIONS OF COMMON TERNS EXPRESSED AS A
PERCENTAGE OF TOTAL EGGS LAID

	Location of colony		
	Buffalo Lake Alberta 1972 ^a	Coquet Island Northumberland 1965 ^b	Bird Island Massachusetts 1971 ^c
	n = 456	n = 580	n = 103
Hatched	61***	88	97
Disappeared	5***	0	0
Cracked or dented	4*	1.5	0
Embryo failed	21***	3	2
Abandoned	6	5	0
Fledged young	28***	52	71

^a This study, ^b Langham (1968), ^c Nisbet (pers. comm.).

* = $P < 0.05$; *** = $P < 0.001$; test for equality of 2 percentages (Sokal and Rohlf 1969).

of colonies on Coquet Island, Northumberland, and coastal Massachusetts, respectively. Their findings are presented in Table 1 as an arbitrary standard with which to measure the relative nesting success of the Buffalo Lake terns. The reproductive effort of Buffalo Lake terns was significantly less successful than these arbitrary standards. The poor success was largely the result of embryonic failure, egg disappearance, and egg damage.

Embryonic failure was responsible for at least 53% of the eggs which failed to hatch. Sixty-one % of these were added, 17% died during the first week, 4% during the second, and 18% during the third week, including 14% which died while pipping. This distribution differs significantly from a constant rate ($P < 0.005$, χ^2), which might be expected if disturbance was the cause. Embryonic mortality and/or infertility occurred in 15–17% of eggs laid by Arctic Terns (*Sterna paradisaea*) nesting on Machias Seal Island, New Brunswick (Pettingill 1939, Hawksley 1957). Embryonic failure was observed in less than 5% of 868 eggs produced in recent nestings of Caspian Terns (*Hydroprogne caspia*) in Finland (Soikkeli 1973).

Thirteen % of eggs which failed to hatch disappeared. Gulls were repeatedly driven from the vicinity of the tern colony. The few eggs which were destroyed by the gulls were located in peripheral nests. Unincubated single eggs (dump eggs) were laid in all areas of the colony and remained intact as long as 28 days. This suggests that it was not unattended eggs which disappeared. The stage of incubation at which eggs disappeared differed significantly from random ($P < 0.025$, χ^2). Forty-eight % disappeared during the last week of incubation and another 36% after they had been incubated for at least the modal

incubation period of 21 to 22 days. The distribution is very similar to that of eggs which were broken. Seventy-two % of broken eggs showed signs of microbial decomposition (malodor, discoloration, and abnormal consistency). Cracking and microbial decomposition were not independent ($P < 0.005$, χ^2). Hence, there was an association among microbial decomposition, cracking, and egg breakage, and a similarity in timing and circumstances under which eggs disappeared or were broken. This suggests that nonviable and/or cracked eggs were detected by the parents and were broken (and eaten?) at the nest, or more frequently, carried away. This suggestion is supported by recent studies of DDE-fed Black Ducks (*Anas rubripes*) (Longcore and Samson 1973) and wild European Sparrowhawks (*Accipiter nisus*) (Newton 1973).

SHELL QUALITY AND ITS RELATIONSHIP TO NESTING SUCCESS

Shell quality, egg weight, and lipid content decrease in successive eggs of the clutch in the Common Tern (Dunn 1972a). Hence, any toxicant-induced variability in shell quality is superimposed upon natural individual and intra-clutch variability. This natural variability may mask any correlations resulting from the effects of toxicant-induced variability if eggs are considered independent of laying sequence.

Eggs were subdivided on the basis of their sequence within the clutch (*A*-, *B*-, and *C*-eggs) and were placed in 1 of 7 beta-backscatter (BBS) classes of equal width. Each class was ranked in the order of increasing shell quality. The various components of nesting success listed in Table 2 were calculated for each BBS class as percentages, and the values ranked on an ascending ordinal scale. The relationship of BBS to the components of nesting success was determined by comparing the rank of the component with the rank of the BBS class using Spearman's rank correlation coefficient (Table 2). As *A*-eggs are present in all clutches, and are usually of the highest quality, they were considered separately. The percentage of eggs with defects, and the percentage of eggs laid which disappeared were negatively correlated ($P < 0.05$) with shell quality, while the percentage of incubated embryos which hatched was positively correlated ($P < 0.01$) with shell quality. No correlation between shell quality and chick survival existed. Eggs which hatched had significantly ($P < 0.001$, Mann-Whitney U test) higher BBS values than those which were dented, cracked, or which disappeared, or in which the embryo died.

EGGSHELL CHARACTERISTICS WHICH ACCOMPANIED EMBRYONIC FAILURE

Shell thickness.—The distribution of thickness indices of the 87 eggs collected during this study was significantly different from the pre-1945 sample ($P < 0.01$, Kolmogorov-Smirnov 2-sample test, Fig. 1). Clearly, the thick-

TABLE 2
CORRELATION BETWEEN SHELL QUALITY AND SUCCESS OF EGGS OF
KNOWN LAYING SEQUENCE

		BBS Classes (ranked in order of increasing shell quality)							Spearman Rank Correlation Coefficient
		1	2	3	4	5	6	7	
Percent of									
Eggs with defects ¹	A-eggs ²	38.5	16.7	38.2	31.2	13.9	0.0		- 0.829*
	All eggs ³	21.9	30.4	32.7	25.6	15.5	9.0	0.0	- 0.750*
Eggs laid which disappeared	A-eggs	23.1	12.5	3.6	4.2	0.0	0.0		- 0.900*
	All eggs	12.5	7.6	5.4	5.8	0.0	0.0	0.0	- 0.857*
Incubated embryos which hatched	A-eggs	57.1	82.3	78.9	93.9	94.1	100.0		+ 0.943**
	All eggs	78.3	82.7	85.5	94.9	89.6	100.0	100.0	+ 0.956**
Chicks hatched sur- viving 14 days	A-eggs	75.0	42.8	53.3	61.3	71.9	41.2		- 0.428
	All eggs	50.0	41.9	41.5	50.0	63.5	45.8	20.0	- 0.223

¹ Infertile, rotten, dented, or cracked.

² 1st egg laid, n = 195.

³ A + B + C eggs, n = 416.

* = P < 0.05; ** = P < 0.01.

ness indices of the eggshells collected during this study are not normally distributed, hence the median is the appropriate measure of central tendency. The median pre-1945 thickness index is greater ($P < 0.0001$, median test) than that of the 1972 sample. The mean thickness index of the 1972 sample (omitting 3 anomalously very thick shells) was 0.889, 3.8% lower than the pre-1945 mean of 0.920. The mean thickness index of dented shells was 13.5% less ($P < 0.025$, Mann-Whitney U test) than the pre-1945 mean.

Shell chemistry.—The avian eggshell is composed primarily of calcium carbonate in the form of calcite (Heyn 1963, Simkiss and Taylor 1971). However, a large number of elements are present in trace quantities, some of which exist as impurities while others, including phosphorus and magnesium, are present in the form of salts. The chemical and physical characteristics of 28 shells collected in the Buffalo Lake colony in 1972, and 3 collected prior to the introduction of DDT, are summarized in Table 3. Although the calcium content of shells of eggs in which the embryos died did not differ from those of viable and pre-1945 eggs, their molar phosphorus concentration was 3 times higher. This increased phosphorus content is sufficient to bind calcium ions in the form of tricalcium phosphate or hydroxyapatite in equal numbers to those combining with carbonate ions to form calcium carbonate under normal conditions. The substitution of PO_3^{-3} for CO_3^{-2} ions in the calcite lattice has the following deleterious structural effects: (1) it distorts the calcite crystal because the foreign metaphosphate ions fit the crystal in

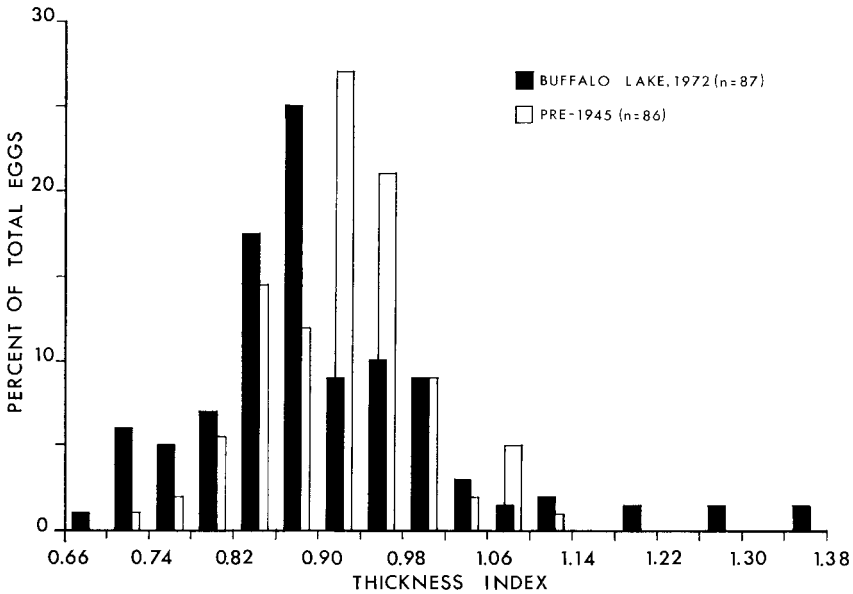


FIG. 1. Distribution of thickness indices of Common Tern eggshells collected in the Buffalo Lake colony in 1972 and of a pre-1945 sample from the Canadian Prairies.

2 dimensions but not in the third, and (2) it removes the electrostatic potential for the absorption of another layer of calcium ions, limiting crystal growth (Simkiss 1964). Dented shells were significantly thinner and weighed 13.3% less per unit surface area than shells of viable eggs but did not differ in their calcium content. Their increased phosphorus content may have been in part responsible for their reduced thickness and strength, and their higher Mg:Ca ratio may have increased brittleness (Brooks and Hale 1955).

Shell structure.—The calcified shell lies immediately external to the shell membranes. Its innermost or mammillary layer consists of roughly hemispherical knobs in the center of which there is an organic core of matrix substance representing the initial nucleation site of crystal growth. Fibers of the outermost shell membrane pass through these cores and firmly attach the membrane to the shell. Mammillae increase in diameter with distance from the membranes and ultimately fuse. The mammillary layer is continuous with the palisade or spongy layer which comprises most of the shell's thickness. The palisade layer has the mammillary bodies as its nucleation sites and thus the crystals are arranged in columns whose junctions are plainly visible in tangential section. Channels arise from some of the spaces between mammillae and pass through the palisade layer and open onto the outer shell

TABLE 3
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SHELLS OF COMMON TERN EGGS

n	Pre-1945	Present Study		
		Living Embryo	Dead Embryo	Dented
	3	12	11	5
Thickness, μ	200	197	205	175*
Thickness index	0.933	0.852	0.947	0.807*
Ca, mg/cm ²	13.18	10.54	11.34	10.80
Percent Ca	39.08	34.68	34.14	37.78
Percent Mg	1.80	1.54	1.43	2.10*
Percent P	0.27	0.24	0.86*	0.63*
Ca : P, molar	111.1	111.1	30.8*	45.5*
Mg : Ca, molar	0.076	0.073	0.069	0.092*

* Mean significantly different from shells containing living embryos ($P < 0.05$, t-test). Pre-1945 eggs not included in statistical comparisons.

surface in small depressions. These pores make gaseous exchange between the embryo and the atmosphere possible. The outer surface of the shell is covered with an organic cuticle. See Becking (1975) for a more detailed description of eggshell structure.

The differences in structure between shells of 10 eggs in which the embryo died and 4 from which chicks hatched are illustrated in Figs. 2-4. During the latter stages of normal embryonic development, calcium is withdrawn from the shell. This occurs at the mammillary cores, hence the eroded central depressions in the bases of the mammillae in Fig. 2A. This decalcification does not alter the number of mammillae, their size, or distribution, nor does it alter the number of pores. Egg failure was accompanied by a generally less-organized structure. Non-viable eggs had 21% fewer ($P < 0.025$, Mann-Whitney U test) mammillae per microscopic field and this decrease in number was accompanied by marked variation in size and shape. Pore openings visible on the inner surface were reduced by 44% ($P < 0.05$, Mann-Whitney U test). The columns, which normally fit tightly together, had irregular-shaped gaps between them, composed of crystals of varying orientation which appeared to replace pores in some cases (Fig. 3D). The palisade layer contained larger, more numerous globular inclusions (Fig. 3B), and in the case of thin shells, was markedly reduced in thickness.

An egg which collapsed on laying showed little development of the palisade layer, and consisted primarily of mammillae which were barely fused together (Fig. 4A). The shell showed normal pigmentation and thus was probably not extruded prematurely. The contents of this egg were severely desiccated 3 days after laying. Another egg, the apex of which was dented inwards

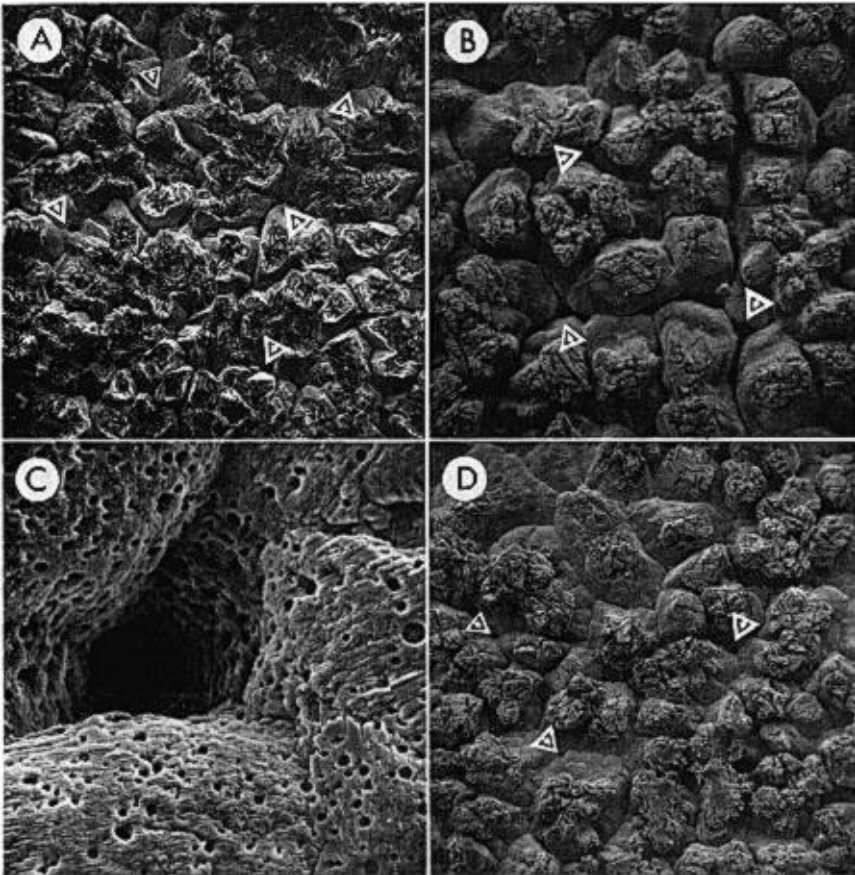


FIG. 2. Structure of Common Tern eggshells collected during this study. A, Inner surface of a viable egg with membranes removed ($168\times$). Note the densely packed, evenly distributed mammillae and prominent pores (arrows). B and D, Inner surface of a non-viable egg with membrane removed ($168\times$). Mammillae are larger, unevenly distributed, and less densely packed. Pores are fewer and less prominent. C, Outer surface of a viable egg with cuticle removed showing a normal pore ($1690\times$).

upon laying, was also deficient in the palisade layer but complete fusion was attained (Fig. 4B). Fusion was attained rather close to the surface making the shell inherently weak. The number and distribution of mammillae was normal as was the external surface. Several eggs collected in this study showed globular projections on the outer surface (Figs. 4C and D), similar to those associated with chlorinated hydrocarbon contamination (Erben and Krampitz 1971, McFarland et al. 1971, Peakall et al. 1973).

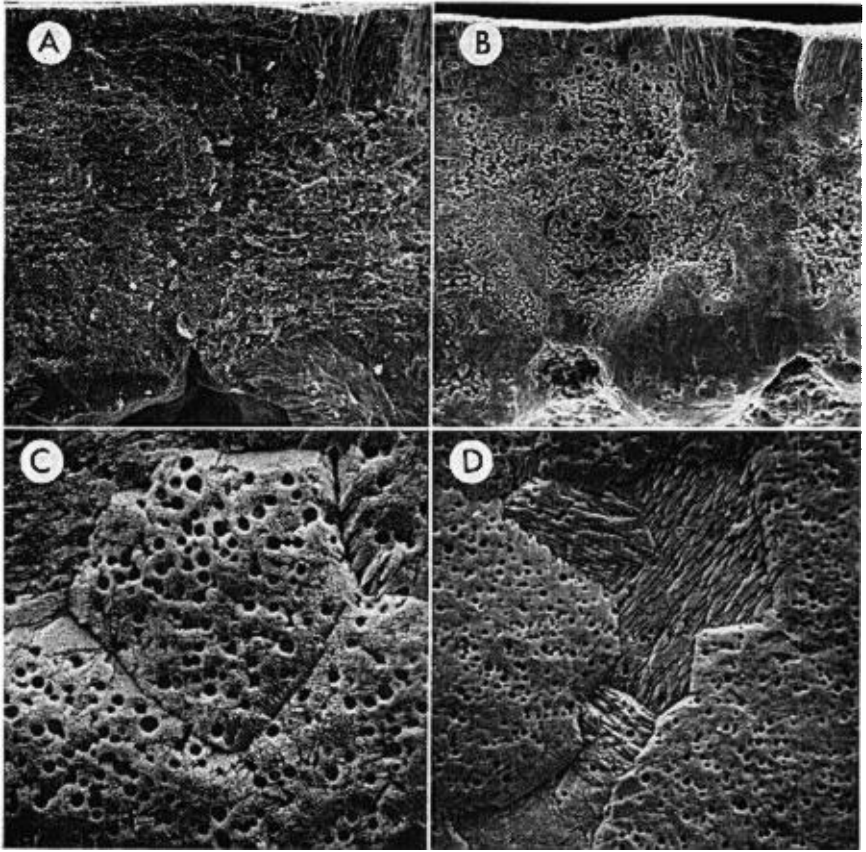


FIG. 3. Structure of Common Tern eggshells collected during this study. A, Radial view of broken edge of a viable egg (770 \times). Note tightly packed mammillae and even, spongy appearance. B, Same view of a non-viable egg (420 \times). The mammillae are less densely packed, and the palisade layer is disorganized and cavitated. C, Tangential view of the outer surface of a viable egg with cuticle removed (1680 \times). Note the close-fitting, regular-shaped junctions between columns. D, Same view of a non-viable egg (840 \times). Junctions between columns are irregular in shape and widely separated with prominent "plugs" of material showing varied crystalline orientation.

A reduction in number, irregular distribution, and increases in the size of mammillae or number of globular inclusions in the palisade layer are accompanied by reduced shell strength (Robinson and King 1970, Simons 1971). Peakall et al. (1973) induced changes in structure and breaking strength in Pekin ducks (*Anas platyrhynchos*) by feeding DDE. Longcore et al. (1971a) fed DDE to Black Ducks and observed an increase in the Mg:Ca ratio and

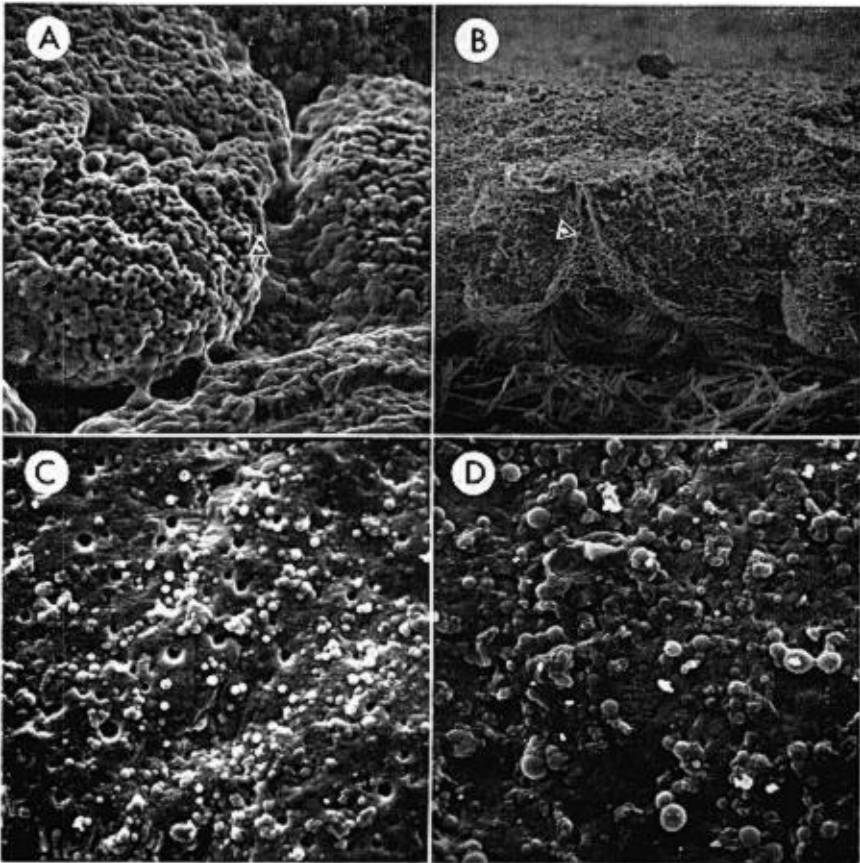


FIG. 4. Structural abnormalities in Common Tern eggshells collected during this study. A, Oblique view of outer surface of a shell which collapsed on laying (1680 \times). Note the prominent fissures between mammillae and absence of palisade layer. B, Oblique view of outer surface of a shell whose apex dented inward upon laying (840 \times). Note the poorly developed palisade layer, disorganized structure, and poor fusion. C and D, Views of the outer surface of two non-viable eggs (1680 \times) showing abundant globular projections which have been associated by other authors with organochlorine contamination.

a marked incidence of cracking of the eggshells. Erben and Krampitz (1971) concluded that the obvious abnormalities in recent Brown Pelican (*Pelecanus occidentalis*) eggshells were an increase in the number of globular inclusions and changes in the amino acid composition of the matrix substance, while Gould (1972) detected changes in the calcite:vaterite ratio. The matrix composition may determine what "morph" of calcium carbonate is deposited during biomineralization (Watabe and Wilbur 1960). Changes in amino acid

composition of the matrix substance, which alter the net charge, could alter the number and distribution of nucleation sites, hence altering the number and distribution of mammillae.

Pore area and gaseous exchange.—Continuous water loss by the egg depends on the relative humidity of the microenvironment and the permeability of the eggshell to water vapor which is determined by the pore area (Wangenstein et al. 1970/71). The measurement of evaporative water loss during normal incubation can be used as a measure of pore area and gaseous exchange (Ar et al. 1974). The mean evaporative water loss of 26 eggs which were incubated but failed to hatch was 2.3 mg/cm² per day. This is significantly ($P < 0.0003$, Mann-Whitney U test) less than the mean of 3.8 mg/cm² per day attained by 15 eggs which hatched. This 39% difference implies a decrease in total pore area. Examination with the scanning electron microscope confirmed a marked decrease in pore numbers. Reductions in pore area or atmospheric oxygen concentration markedly reduce the hatchability of chicken (*Callus gallus*) eggs (Taylor et al. 1956, Tazawa 1972, Wangenstein et al. 1974). Peakall et al. (1973) observed marked decreases in pore area of eggshells of DDT-fed American Kestrels (*Falco sparverius*), Ringed Turtle Doves (*Streptopelia risoria*), and Pekin ducks.

ORGANOCHLORINE RESIDUES

Forty-four eggs were analysed for organochlorine residues. Thirteen eggs were selected at random from the total available and were analysed individually. The remaining 31 were analysed in 5 pools of 5, and 2 pools of 3; each pool containing eggs with a common chemical or physical shell characteristic. This sample represents about 10% of the eggs laid and is biased towards eggs which failed to hatch or which were obviously abnormal. DDE and PCB's were present in all samples, dieldrin was present at trace levels. PCB's were present at approximately $\frac{1}{10}$ the concentration of DDE but were not quantified. The mean DDE content of these 44 eggs was 3.98 ppm wet weight. A range of 0.05 to 11.08 ppm was encountered in the 13 individual eggs (geo. mean = 1.54 ppm). A pool composed of 5 eggs with dented shells, whose mean thickness index was 17% less than the pre-1945 mean, contained 6.67 ppm DDE (wet weight basis), almost twice the mean value of 3.42 ppm detected in the other 39 eggs ($P < 0.01$, modified t-test).

B. Switzer and colleagues (pers. comm.) found a highly significant negative correlation between shell thickness and DDE residues of 40 randomly collected eggs from a nearby colony. The mean DDE content of their random sample was 4.42 ppm wet weight, in contrast to 7.72 ppm in 13 eggs with dented shells ($P < 0.01$). The dented shells were 12% thinner than the random sample ($P < 0.01$). A DDE:PCB ratio of 9:1 was found in these eggs.

A pool of 10 food items collected from those brought to the colony, and constructed to represent the relative proportion each species contributed to the diet of the terns while present on Buffalo Lake, contained 0.02 ppm DDE. PCB's and dieldrin were absent. It is apparent that the body burden of DDE and PCB which is reflected in the eggs of Buffalo Lake terns is not accumulated on the breeding grounds. Recoveries of 8 terns banded in Alberta colonies indicate that they winter and spend the first 2 years of their life along the coast of Southern California (5), Mexico (1), El Salvador (1), and Peru (1). The coast of southern California is heavily contaminated with organochlorines (Burnett 1971, Risebrough et al. 1967). DDE:PCB ratios as high as 9:1 have been associated only with the Gulf of California. Hence it is likely that this is the site of contamination of the Alberta population.

Five eggs collected from the highly successful Coquet Island colony in 1965 contained a mean of 0.04 ppm DDE and food fish contained 0.03 to 0.08 ppm (Robinson et al. 1967). Thus, Buffalo Lake tern eggs contained 95 times more DDE than those of Coquet Island terns, whereas the levels in food fish in the areas of the 2 colonies were similar.

Eggshell thinning, increased egg breakage and/or disappearance, increased embryonic mortality, and decreased hatchability have been observed in controlled laboratory studies of American Kestrels (Porter and Wiemeyer 1969), Black Ducks (Longcore et al. 1971b), and Bengalese Finches (*Lonchura striata*, Jefferies 1971) fed low dietary levels of DDE. Studies of American Kestrels and Pekin ducks have shown that carbonic anhydrase levels in the shell glands vary inversely with dietary DDE and directly with shell thickness (Lincer 1972, Pritchard et al. 1972). Field studies of European Sparrowhawks (Newton and Bogan 1974) clearly show the relationship of shell thinning and organochlorine contamination to egg breakage, embryonic mortality, and hatching success.

SHELL QUALITY AND ITS RELATIONSHIP TO BEHAVIOR

No difference was detected in the tendency to retrieve an egg from the nest rim between parents who produced eggs with high and low quality shells. This is an indication that no difference in the intensity of incubation instinct accompanied the variation in shell quality observed. Enderson et al. (1973) detected no abnormalities in films of parental behavior of unsuccessful tundra Peregrines (*Falco peregrinus*). Peakall and Peakall (1973) present evidence that altered incubation rhythm resulted in increased embryonic mortality in PCB-fed Ringed Turtle Doves.

Seventeen tern chicks, 3 to 8 days of age, representing eggs from 17 clutches with high shell quality (BBS classes 5, 6, and 7) were tested on a visual cliff. Fourteen (82%) reacted to the visual situation, 13 (93%) of them chose the

shallow side. In contrast, of 14 chicks of similar age representing 14 clutches with low shell quality (BBS classes 1 and 2), only 6 (43%) reacted to the visual situation and only 4 (66%) chose the visually shallow side. This behavioral difference was independent of fresh egg weight and weight or age of the chick at testing. The difference in the proportion of chicks reacting to the visual situation is marginally significant ($0.10 < P > 0.05$, χ^2), and indicates a difference in motivation. The difference in the proportion of chicks choosing the visually shallow side is significant ($P < 0.05$, Fisher test) and is indicative of altered depth perception. Nisbet (pers. comm.) tested 150 chicks in the Massachusetts colonies in 1973, using identical equipment, and found 93% of those which responded chose the shallow side. The majority of his chicks reacted to the stimuli. The normal feeding pattern of tern chicks requires that they recognize the presence of a fish in the parent's beak and accurately and actively peck at the head rather than the tail of the fish (Quine and Cullen 1964). Hence good visual and depth perception are necessary. No differences in chick survival or growth could be associated with visual cliff performance. Survival and growth of C-chicks of Common Terns were positively correlated with the feeding performance of the male parent (Nisbet 1973). Terns are sight-oriented, plunge-diving predators and as such require high acuity and good depth perception (Dunn 1972b, Salt and Willard 1971). Hence altered visual perception, if it persists, could reduce an individual's feeding efficiency and thus reduce its chances of survival and lower its performance as a parent.

The feeding of dieldrin (Baxter et al. 1971) and PCB (Dalgren and Linder 1971) to penned pheasants (*Phasianus colchicus*) altered the visual cliff performance of their chicks. Revzin (1966) found that endrin, another organochlorine, selectively affects the avian ectostriatum, the visual projection area of the telencephalon. Levels required to produce perceptual deficits are much lower than those needed to produce overt behavioral disturbances. Altered visual perception of tern chicks in this study is likely the result of the presence of a toxicant in the egg contents, a toxicant which is capable of altering shell quality.

CONCLUSION

Although the rigidity, calcium content, and antimicrobial properties of the shells of the majority of eggs which failed to hatch did not differ from those of viable eggs, abnormalities in shell structure and composition were responsible for shell damage, which resulted in egg disappearance, and for embryonic death through hypoxia. Reduced pore area was not associated with any significant thinning.

Various chemical and structural abnormalities similar to those observed

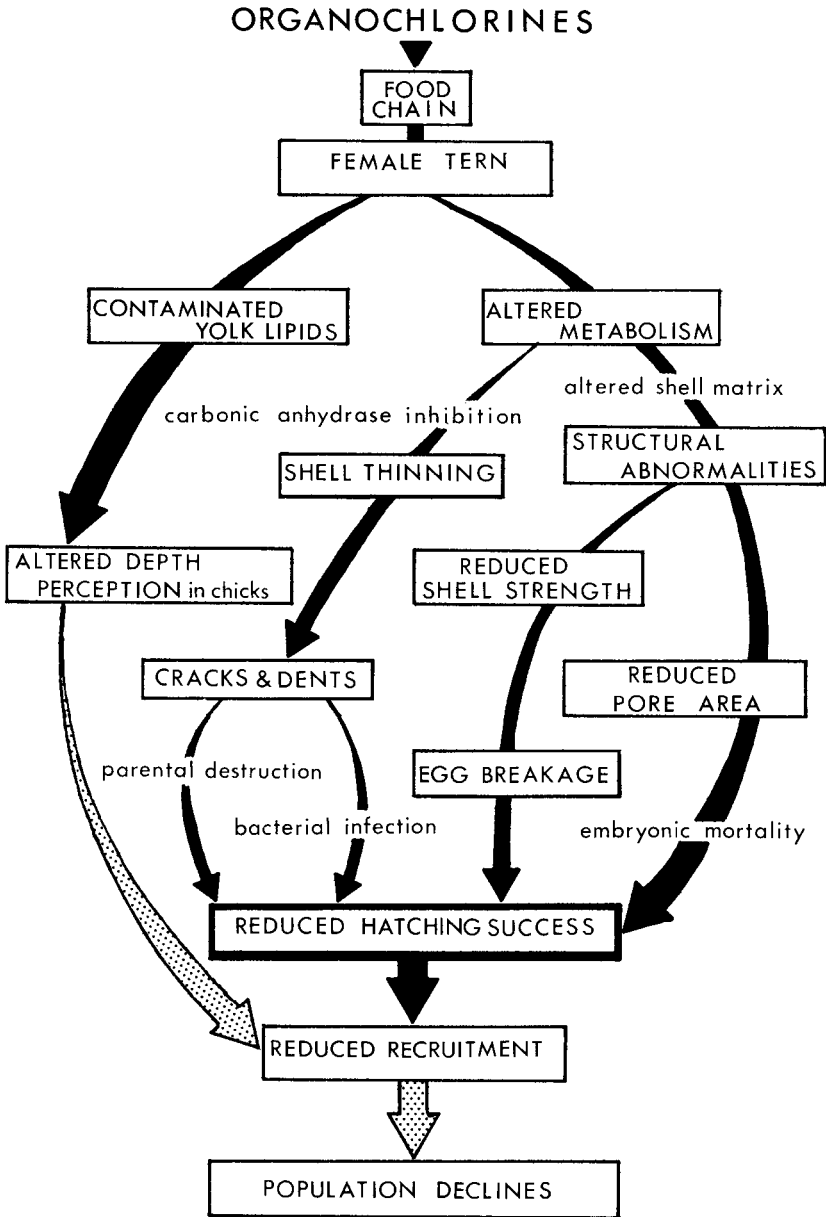


FIG. 5. The mechanisms and interrelationships by which organochlorines are thought to affect reproductive success in the Common Tern. Solid arrows indicate mechanisms which were observed in this study and shaded arrows represent postulated outcomes.

in this study have been induced in several species by incorporating DDE into their diet. Thus, there are several ways that organochlorines, and in particular DDE, can affect eggshells to induce embryonic mortality independent of shell thinning. In the Common Tern these mechanisms are operative at residue levels lower than those associated with marked shell thinning and are probably far more effective in reducing hatching success. Figure 5 is an attempt to indicate the mechanisms and relationships among factors involved in the "reduced hatchability syndrome" as we know them at the present time and as they are seen in the Common Tern. Whether they are functional in all species remains to be determined.

SUMMARY

A portable beta-backscatter gauge was used to measure the shell quality of 416 eggs laid in a colony of Common Terns located on Buffalo Lake, Alberta, Canada (52° 20' N, 112° 50' W) in 1972.

Eggshell quality as measured by beta-backscatter (a composite response to thickness, chemical composition and ultrastructure) was an important determinant of hatching success. Abnormalities in shell structure and chemical composition were responsible for shell damage, which resulted in egg disappearance, and for death through hypoxia. There were also indications that low levels of DDE contamination had detrimental effects on the visual perception of chicks. No difference in intensity of incubation instinct could be detected between parents who produced eggs with high and low quality shells.

Eggshells had a mean thickness index which was 3.8% lower than the pre-1945 mean for this geographical population. Denting of shells accompanied a 13.3% decrease in the thickness index which was not accompanied by a significant decrease in calcium content, but by increased molar Mg:Ca ratio. Both dented shells and shells of eggs in which the embryo died had significantly increased phosphorus contents when compared to shells surrounding viable embryos. Although small size of the pre-1945 sample prevented statistical comparisons, the chemical and physical characteristics of shells surrounding viable embryos were very similar to pre-1945 shells. Scanning electron microscopy revealed that shells of eggs in which embryos died had 44% fewer pores, and 21% fewer mammillae which were larger and more irregular in their distribution than those of shells of eggs which hatched. Evaporative water loss from eggs which were incubated but failed to hatch was 39% less than that from eggs which hatched, reflecting the reduction in pore numbers.

The mean DDE content of 39 eggs with nondented shells was 3.42 ppm wet weight, while 5 eggs with dented shells contained a mean of 6.77 ppm. PCB's were present at about $\frac{1}{40}$ the concentration of DDE. Food fish from Buffalo Lake contained a mean DDE content of 0.02 ppm wet weight basis. Hence, contamination is assumed to occur on the wintering grounds.

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