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THE UNIVERSITY OF ALBERTA

EGGSHELL QUALITY AND DDE: CORRELATION WITH REPRODUCTIVE SUCCESS IN THE COMMON TERN

by

© GLEN ALLEN FOX

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Eggshell quality and DDE: Correlation with reproductive success in the common tern" submitted by Glen Allen Fox in partial fulfilment of the requirements for the degree of Master of Science.

W. C. Mackay
Acting Supervisor

Date February 26 1974

Gratefully dedicated to

Stuart Houston, Alex Dzubin, Fran Hamerstom, and Sandy Middleton--

my mentors, teachers, and friends

ABSTRACT

A portable gauge based on the beta-backscatter principle was characterized and used to measure the shell quality of 456 eggs laid in a colony of common terns, *Sterna hirundo* L., located at Buffalo Lake, Alberta, in 1972. The nesting success of this colony was significantly lower than that attained by other populations of this species. The low nesting success resulted from higher rates of egg disappearance, egg damage, and embryonic death.

Hatchability was positively correlated with shell quality. Egg disappearance and egg damage were negatively correlated with shell quality. Chicks from eggs with low quality shells had poorer depth perception or were less motivated by stimuli provided by the visual cliff than chicks which hatched from eggs with high quality shells. Denting of shells accompanied an increase in magnesium and phosphorus content and a decrease in thickness which was not accompanied by a decrease in calcium content. Shells in which embryos died had fewer and larger mammillae which were more irregular in their distribution, and fewer pores than shells of viable eggs. Evaporative water loss of eggs which failed was 38 percent less than from eggs which hatched, reflecting a reduction in pore area.

The mean *p,p'*-DDE content of 44 eggs collected from the colony in 1972 was 3.80 ppm w/w (0.50-11.08). Food items contained a mean of 0.02 ppm. Egg DDE residues were negatively correlated with the thickness index of the shell. Dented eggs contained a mean of 6.77 ppm DDE and had a thickness index which was 17 percent less than the mean for a pre-DDT sample.

A review of recent literature indicates that DDE has been associated with increased rates of egg disappearance, egg damage, embryonic failure, similar abnormalities in shell structure and evaporative water loss, and abnormal depth perception in declining populations of raptorial or piscivorous species and/or in laboratory studies. It is suggested that DDE-induced abnormalities in shell structure and composition are responsible for shell damage, which results in egg disappearance, and for embryonic death through respiratory failure. Hence, a mechanism of pathogenesis in addition to carbonic anhydrase inhibition is proposed which is active in the presence of very low levels of DDE, and which appears to be responsible for reducing the reproductive success of the common terns nesting at Buffalo Lake.

A comparison with causes of reduced reproductive success and chemical and structural characteristics of pre-DDT and recent eggshells of the American merlin, *Falco columbarius*, indicates that the relative importance of various causes of reproductive failure differs between terns and falcons. Eggshell thinning and resulting egg damage appear to be the major cause of reduced reproductive success in falcons. No marked structural abnormalities accompanied this shell thinning. However, shell thinning of falcon eggs was accompanied by a marked decrease in calcium content.

There would appear to be interspecific variation in sensitivity to DDE, and in the mechanism of pathogenesis. This may account for the variability in results of field investigations and must be considered when predicting the outcome of reduced environmental contamination.

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INTRODUCTION

Chlorinated hydrocarbons, particularly *p,p*'-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene) and its environmentally widespread and more stable metabolite *p,p*'-DDE (1,1-dichloro-2,2,-bis(*p*-chlorophenyl) ethylene) have been implicated in recent decreases in relative weight of eggshells of bird- and fish-eating predatory birds, and the accompanying decrease in their nesting success (Anderson and Hickey, 1972; Blus *et al.*, 1971; Cade *et al.*, 1971; Gress *et al.*, 1973; Lincer, 1972; and Ratcliffe, 1970). To date there have been no studies of the relative importance of the abnormal shell, the DDE content of the yolk lipids, and parental behavior in these decreases in nesting success.

The common tern, *Sterna hirundo* L., is a common colonial-nesting, piscivorous species which is abundant in Alberta. The colonial situation offers the opportunity to examine a large number of eggs produced by genetically related individuals (Austin, 1949, 1951) subjected to a common set of proximate environmental variables. Eggs of this species collected from Alberta colonies contain levels of DDE that are comparable to those observed in some declining species (Switzer *et al.*, 1971; Vermeer and Reynolds, 1970). The nesting success of common terns nesting in recent years at Chip Lake, Alberta, has been low (Switzer *et al.*, in press), but there is no evidence of a population decline.

The objectives of this study were to determine: (1) how the nesting success of an Alberta colony compares with that attained by other populations of this species; (2) if any relationship exists between eggshell quality and nesting success in common terns;

(3) what chemical, structural, and physiological characteristics of the eggshell accompany egg failure: and (4) if any variation in parental or chick behavior accompanies variation in eggshell quality.

The avian eggshell serves as a rigid container, a microbial barrier (Board and Halls, 1973; Walden *et al.*, 1956), the source of skeletal calcium (Simkiss, 1961), and the principal resistance to gaseous exchange (Wangensteen *et al.*, 1970/71) for the developing embryo. Changes in any of these parameters could conceivably influence hatchability.

The eggshell is composed of an inner and an outer shell membrane, and the calcified shell. The formation of the eggshell is initiated in the isthmus region of the oviduct where the shell membranes are secreted by the tubular glands. The nucleation sites of the biomineralization process, the mammillary cores, are thought to be formed in the isthmus region, whereas calcification occurs in the shell gland (Simkiss and Taylor, 1971). The cells of the gland secrete carbonic acid into the lumen where it can combine with diffusible calcium ions derived from medullary bone to form calcium carbonate in the form of calcite. Hence, decreased shell weight reflects decreased calcium carbonate content. Carbonic acid formation involves catalysis by the enzyme carbonic anhydrase. Specific inhibition of this enzyme results in thin-shelled eggs (Gutowska and Mitchell, 1945) and decreased shell weight (Bernstein *et al.*, 1968). Carbonic anhydrase levels in the shell glands of captive American kestrels *Falco sparverius* varied inversely with dietary DDE and directly with shell quality (Lincer, 1972). Pritchard *et al.* (1972) examined the components of the calcium carbonate-forming mechanism in the shell gland mucosa of DDE-dosed pekin ducks *Anas platyrhynchos*. They found no change in the calcium-binding protein and ATPase activity, but a dose-response relationship existed between the degree of carbonic anhydrase inhibition and the level of mucosal DDE. Purified avian carbonic anhydrase is distinct from other carbonic anhydrases in certain of its chemical properties which may reflect its sensitivity to chlorinated hydrocarbons (Bernstein and Schraer, 1971; Serine and Schraer, 1972).

The bulk of the evidence, both from laboratory and field studies, implicating chlorinated hydrocarbons in eggshell thinning and decreased reproductive success, has been based on the determination of residues and shell quality values in a sample of eggs (in some cases only those which failed to hatch), and relating these to the reproductive success of the population by making comparisons with those values obtained for other populations. To date, no one has published a study of the fate of individual eggs of known shell quality in a single population. Such a study necessitates the use of a non-destructive method of measuring eggshell quality in the field. The beta-backscatter method designed by James and Retzer (1967) is such a technique.

Backscattering is a simultaneous response to three distinct properties of the target: the mass of the constituents, their depth distribution, and the crystalline structure (Nicolet *et al.*, 1972). Hence, in a gauge based on the beta-backscatter principle, the response is to the quantity of shell material present, and does not differentiate between variation in density and variation in thickness. Studies of variation in shell characteristics among domestic fowl indicate that beta-backscatter (BBS) correlates highly with shell thickness and strength as measured by impact resistance (Wilson *et al.*, 1968) and non-destructive deformation (Hunton, 1969).

STUDY AREA AND BASIC BIOLOGY OF THE TERNS

The colony chosen for study was located on an island in Buffalo Lake, Alberta (52° 20' N., 112° 50' W.), approximately one kilometer from Rochon Sands Provincial Park. The island is approximately one hectare in area and has a maximum elevation of 1.5 meters above water level. The terns occupied a sandy area approximately 0.25 hectare in extent at one end of the island. The substrate of the remaining area did not appear to be suitable tern habitat and was occupied by some 1,000 pairs of gulls, *Larus delawarensis* and *L. californicus*. Hence the gull and tern colonies were spatially separated. The island was frequently visited by the public but signs placed at strategic locations were very effective in reducing human disturbance.

When the colony was first visited on May 11, 1972, the lake was still covered with slush ice and only five terns were observed. On May 20, seven terns were seen but no nesting scrapes were located. Three eggs and some 100 terns were observed on May 24. Laying continued through July 13. An increase in the number of terns in the colony was observed between June 7 and 14. The first chick hatched on June 16 and the first flying young were observed on July 17. Downy young were observed as late as August 16. A decrease in the number of adults was first noted on August 9. On September 10, the island was devoid of terns. A search of the lakeshore located three adults and 15 to 20 flying young. Conversations with local residents indicated that few terns had been seen in the previous week.

Ninety-six food items found in or near nest scrapes or protruding from the mouths of chicks were identified. Burbot, *Lota lota*, usually weighing less than a gram and about 4 cm in length, constituted 77 percent of the food brought to the chicks (frequency basis). Brook sticklebacks, *Culaea inconstans*, accounted for 12 percent and spottail shiners, *Notropis hudsonius*, an additional 4 percent. The remainder consisted of dragonfly nymphs (Odonata) and a chorus frog, *Pseudacris triceriata*. No differences were noted between the composition of food items brought to the chicks and those brought to incubating individuals or captured and eaten by hunting adults.

MATERIALS AND METHODS

BETA-BACKSCATTER GAUGE AND COUNTING PROCEDURE

The original design of James and Retzer (1967) was the basis for the gauge used in this study. However, the original design was not operable in the field. Mr. F. Anderka of Custom Electronics, Ottawa, designed and built the entire portable version. The egg holder, which contains both the radioactive source and the Geiger-Mueller detector, was fabricated as specified by the U.S.D.A. (1968). The radioactive source was 10 microcuries of Ruthenium 106 and was fabricated by H. Pressly of the Oak Ridge National Laboratory, U.S. Atomic Energy Commission. The finished gauge consisted of the counting device—which included a 1, 2, 4, 8, and 16 second timer, a six-digit decade counter, and a test circuit; the power source—a 12 volt rechargeable gelled electrolyte battery; and the egg holder (Figure 1). These three components were fitted into a foam-rubber-lined wooden case with a removable lid. The case provided shelter and shade during the counting operations, and protected the electronic components from shock and the weather during transport. The finished unit was compact, light, highly portable, and functioned well under field conditions (Figure 2c).

The distance between the eggshell and the source is fixed by the design of the holder and thus is constant (Figure 2d). Hence the BBS count reflects the activity of the radioactive source, the degree to which the eggshell being measured reflects (backscatters) the beta particles, and the duration of the count. This necessitated standardization

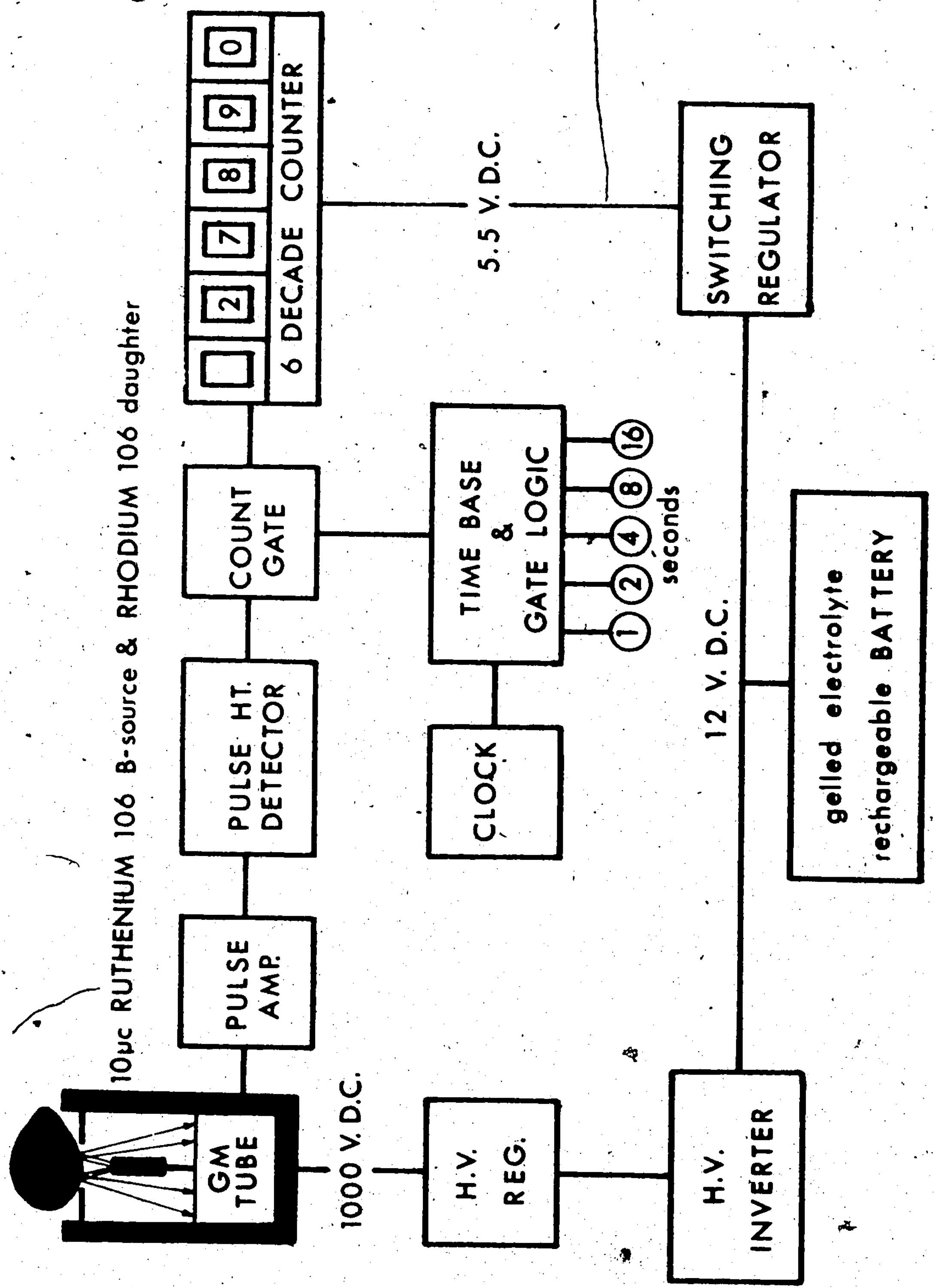


Figure 1. Block diagram showing components of the portable Beta-backscatter gauge used to measure eggshell quality in this study.

Figure 2. (A) Adult Common Tern. (B) A typical dented tern egg. (C) The complete BBS device with egg in counting position. The lid which is used as a standard is in the foreground. (D) Detail of egg-holder showing beta source (*arrow*) and egg supports. (E) Egg-holder with tern egg in counting position. (F) Visual cliff apparatus. Chick is on the visually shallow side and facing away from the "cliff."


A
B
007
C
D
E
F

of the counting procedure. The following procedure was adopted for all BBS counts. At the beginning and end of each counting session, three successive 8-second counts were taken with the metal lid in place on the holder. The lid was chosen as an arbitrary standard. The total count for this standard over the 24-second period was recorded. The counter was then zero'd and three successive counts were taken with the lid removed. The total count over the 24-second period was recorded as the background count. The standard count minus the background count for the counting session was regarded as the "standard value." Eggs or eggshells were placed on the holder so that their waist was centered over the source, counted for 8 seconds, rotated 120°, counted for another 8 seconds, rotated another 120°, and counted for a final 8 seconds. The total count obtained over the 24-second period thus represents the summation of counts for three well-separated points on the circumference of the waist of the eggshell. The total count for the egg was recorded. The total count for the egg minus the background count was regarded as the "egg value." The *relativa BBS value* for the egg represents the ratio of the "egg value" to the "standard value" expressed as a percentage.

FIELD

Forty-four visits were made to the colony between May 20 and August 16, 1972, and a total of 136 hours was spent in the colony. Visits were made, when possible, between 0800 hours and 1100 hours, and were limited to two hours in length. The terns soon became accustomed to the observer and only individuals nesting and resting immediately adjacent to the observer would flush on approach.

Each nest was marked with a 45 cm length of lath. The nests were given consecutive numbers in the order they were found. The nest number was marked on the nest stake and on each egg of the clutch with a felt pen. When known, the sequence of laying was also recorded on the eggs.

Each egg was weighed when fresh to the nearest 0.10 g on a torsion balance and its shell quality measured with the BBS gauge. The colony was searched for new nests on each visit, and the contents of each nest were recorded at 48-hour intervals, weather permitting. All eggs were examined for cracks and dents each time a nest was checked. Eggs which were cold when examined were placed on their large end and if they remained in that position on the next visit, they were regarded as abandoned.

A number of nests were fenced, either individually or in a group with ½" poultry netting. The netting was cut and dug into the ground in such a manner that the fences were 30 to 35 cm high, as recommended by Nisbet and Drury (1972).

Baerends (1969) has recommended egg displacement as a method of determining the intensity of incubation instinct in larids. Special visits were made to the colony, during which an egg was displaced from a number of selected nests. One egg in each of 80 nests was displaced approximately 10 cm from the nest scrape early and late in the incubation period. The observer returned to the colony 30 minutes later and recorded whether or not the eggs had been returned to the nest scrape in the test period.

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

Evaporative water loss of selected eggs was determined by weighing the egg on two or more successive visits. The surface area of the egg was calculated as 4.14 x fresh egg weight$^{.66}$ (g) (based on data presented for the arctic tern, *Sterna paradisaea*, in Drent, 1970). Evaporative water loss was expressed as mg/cm^2 per day.

All young were banded with a Canadian Wildlife Service band when first observed, and every effort was made to ascertain the identity of the egg from which they hatched. Young produced in fenced nests were weighed to the nearest gram with a Pezola spring balance and their tarsus length and wing chord measured to the nearest millimeter with a flexible plastic ruler. These young were weighed and measured at 48-hour intervals, weather permitting, to determine their rate of growth. The identity and condition of all chicks encountered on each visit were recorded.

Fifty-five young were tested on a visual cliff when three to eight days of age. The visual cliff consisted of a 40 cm x 35 cm plexiglass platform over a sliding cliff 30 cm deep, which was painted in a black checkerboard pattern, surrounded by a 15 cm wooden fence (Figure 2f). The testing procedure was to locate the cliff edge in the middle of the field and to place the chick on the plexiglass so it was at the edge of the "cliff." The chick was given three minutes to choose the shallow or the deep side by stepping backward [illegible]orward, respectively. Care was taken to ensure that the chick actually looked down at the floor at least once during the test period. If it did make a choice, the procedure was repeated by sliding the "cliff" into a new position to correspond with the chick's new position. A chick which did not react in three minutes was 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. Older chicks were too aggressive to be tested, whereas chicks of less than 72 hours of age had difficulty supporting themselves on the plexiglass floor. Chicks that were tested on two or more occasions were consistent in their response. No attempt was made to orient the "cliff" in any regular fashion with regard to the sun. However, the cliff was placed in the same location for all tests and orientation was probably relatively consistent.

LABORATORY

Egg Measurements

The length (L) and breadth (B) of the air-dried eggshell were measured to 0.1 mm with a dial caliper. The weight was determined to the nearest 0.001 g on a Mettler top-loading balance, or in the case of eggs in museum collections, 0.01 g on a torsion balance. These measurements were used to calculate the surface area (SA), the thickness index (TI), and weight per unit surface area (mg/cm^2) as follows:

$$\text{SA (cm}^2\text{)} = 4.83\ \frac{(3.14 \times L \times B^2)}{6}^{.66}$$

$$\text{TI} = \frac{\text{weight (mg)}}{L\ \text{(mm)} \times B\ \text{(mm)}} \quad \text{(Ratcliffe, 1967)}$$

$$\text{mg/cm}^2 = \frac{\text{weight (mg)}}{\text{SA (cm}^2\text{)}}$$

A sample (*ca.* 1 cm^2) was cut from the waist of those shells which were chosen for chemical analysis, using an abrasive disc powered by a Dremel Moto-Tool. The average thickness of this sample with attached membrane was determined to the nearest 0.005 mm using a modified Mitutoyo dial micrometer (Lewin, 1970).

Chemical Analysis

The shell sample was weighed to the nearest 0.001 g, boiled in 2.5 N sodium hydroxide until the membranes were released, rinsed in distilled water, and allowed to digest overnight in 10.0 ml of 1.0 N hydrochloric acid.

The magnesium concentration of the undiluted digest was determined with a Jarrell-Ash atomic absorption spectrophotometer using the 285.2 nanometer absorption line and an air-acetylene flame. Calcium was determined in a 1/400 or 1/1,000 dilution of the digest using the same instrument, operating in the emission mode, using the 422.7 nanometer line and nitrous oxide-acetylene flame. Calibration was done using a 1/100, 1/200, and 1,000 dilution of an aqueous stock standard containing 1,000 ppm calcium and 300 ppm magnesium. This stock standard was prepared by dissolving 3.676 g $CaCl_2 \cdot 2H_2O$ and 3.043 g $MgSO_4 \cdot 7H_2O$ in deionized glass distilled water and diluting to 1 liter.

The phosphorus content of the digest was determined by the colorimetric method of Hardwood *et al.* (1969) as outlined in Appendix 1. A Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer and 1.0 cm light-path cuvettes were used to measure the concentration of the resulting colored complex at 890 nanometers.

An eggshell control was prepared by grinding numerous chicken eggshells, which had been previously boiled in 2.5 N sodium hydroxide, to a fine powder. Two hundred and fifty milligrams of this powder was digested in 25 ml of 1.0 N hydrochloric acid. This shell digest was used as a control in all chemical determinations.

Chlorinated Hydrocarbon Residue Analysis

The frozen egg contents were thawed, weighed to the nearest 0.01 g, and homogenized. 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:

$$5.0 \text{ g} \times \frac{\text{weight of total homogenate}}{\text{weight of fresh egg} - \text{weight of shell}}$$

Food items were removed from the 4 percent formalin preservative, placed on paper towels, and the formalin allowed to drain from them. The entire item was used in the homogenate.

The clean-up procedure was that of Bonelli (1966) employing a Florisil column as outlined in Appendix 2. Identification and quantification of residues in the eluate were performed by Mr. W. A. McBlain using a Varian Aerograph 600D gas liquid chromatograph equipped with a 250 microcurie tritium source electron capture detector, and a Varian Aerograph model 20 strip chart recorder. A 1.6 m glass column packed with a 1:1 mixture of 10 percent DC-200 and 15 percent QF1 on 80/90 mesh Anakrom ABS was used for the separation of compounds. The carrier gas was purified nitrogen at a flow rate of 70-85 ml/min. The oven temperature was 175° to 185° C. Quantification was based on comparison of peak heights to those of standards containing multiples of 10^{-8} g/ml of *o,p*'-DDE, *p,p*'-DDE, Dieldrin, *o,p*'-DDT, *p,p*'-DDD, *p,p*'-DDT, and

Methoxychlor, or 10^{-6} g/ml of Aroclor 1254. Only *p,p*'-DDE was quantified as it was the only chlorinated hydrocarbon insecticide present in appreciable quantities in any of the samples. Recovery of a known amount of *p,p*'-DDE from a tern egg was 113 percent and the reproducibility of recoveries was ±0.25 percent. Polychlorinated biphenyls (PCB's) were present in all egg samples but no attempt was made to quantify them. The interference of PCB's prevented the detection of insecticides other than *p,p*'-DDE which could have been present in trace amounts.

Bacteriological Examinations

Bacteriological examination of egg contents consisted of inoculating the thawed egg contents by means of a cotton swab on brain-heart infusion and MacConkey agar plates. The swab was then placed in fluid thioglycollate medium. The plates and broth were incubated aerobically at 37° C. Preliminary identification of organisms was based on colonial morphology, Gram's stain, lactose fermentation and reactions on triple-sugar iron agar and urea agar. Final identification was provided by Dr. M. Finlayson of the Provincial Laboratory of Public Health.

Objects from the environment consisting of soil, debris from the feet of dead chicks, and swabs from the surface of food items were treated in a similar manner.

Scanning Electron Microscopy (SEM)

Small fragments (*ca.* 3 mm^2) were removed from the waist of selected eggshells for SEM examination. Contact cement was used to mount three shell specimens on a single stub. One piece of untreated shell 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 obtain views of the external surface, the internal surface with membranes removed, and the structural elements in sagittal section by examining one stub.

The stubs with mounted eggshells were coated with gold in a vacuum evaporator and subsequently examined with the technical assistance of Mr. George Braybrook, using a Cambridge Stereoscan S4 SEM located in the Department of Entomology of this University.

Photographs of representative and selected portions of these fragments were taken at standard magnifications. Contact prints of the resulting 2¼" x 2¼" images were used for study and quantification. The total number of pore openings and the total number of mammillary bodies present in the representative field of the internal surface were counted. All other observations were of a qualitative nature. Admittedly, there was much variation in the distribution of ultrastructural elements within the total surface area. However, by selecting the sample from the waist of the shell, and by quantifying a representative field rather than an exceptional field, it was hoped that bias would be eliminated and statistical comparisons would be valid.

Calculations and Statistics

Calculations and statistical analyses were performed on an Olivetti-Underwood Programma 101 desk calculator-computer. Programs for the calculation of relative BBS, TI, SA, and various chemical parameters were written by Dr. B. Chernick.

Statistical programs used were those presented in the Programma 101 statistical manual. Two-tailed and unpaired tests were applied in all cases. Unless otherwise stated, the significance level was 0.05. Nonparametric tests were applied whenever the normality of the data was questionable. Critical values were those presented in Roscoe (1969) and Schefler (1969). Comparisons between percentages were made by the method of Sokal and Rohlf (1969:607-608).

CHARACTERIZATION OF THE BETA-BACKSCATTER RESPONSE

It was necessary to determine which of the routinely measured characteristics of eggshells are reflected by the BBS measurement. Correlations between BBS and physical and chemical parameters most commonly measured were calculated for four species representing three orders (Table 1). It is clear that those parameters which have a weight component are highly correlated. Weight is a function of mean thickness and density. Density is a function of chemical composition and concentration. Calcium and magnesium are two of the three major cations present in the avian eggshell. No measurements were made of potassium, the other major cationic component, but the relatively high correlations between BBS and the calcium + magnesium content indicate that the BBS response is predominantly a function of cation content. Thickness was measured to the nearest 0.005 mm, which was the limit of accuracy in the author's opinion. In thin-shelled species (i.e., quail and tern) the apparent lack of response to shell thickness may very well reflect the lack of sensitivity of the thickness measurement and the resulting large number of tied thickness values.

The response of the BBS gauge to interspecific differences in weight of shell per unit area for 12 species representing nine genera in

TABLE 1

CORRELATIONS BETWEEN RELATIVE BBS AND PHYSICO-CHEMICAL MEASUREMENTS OF EGGSHELLS OF JAPANESE QUAIL (JQ), COMMON TERN (CT), AMERICAN MERLIN (AM), AND PRAIRIE FALCON (PF)

Variables	Correlation coefficient (r_s)			
BBS *vs*.	JQ (26)[a]	CT (29)	AM (23)[b]	PF (22)[b]
Thickness, mm	0.195	0.300	0.521**	0.824**
Weight, mg	0.829**	0.861**	0.880**	0.719**
Thickness Index	0.703**	0.962**	0.902**	0.839**
Ca plus Mg, mg/cm^2	0.712**	0.654**	0.547**	0.618**

(Sample size) **$P < 0.01$.

[a] Japanese quail eggs supplied by Laboratory Animal Services, University of Alberta.

[b] Falcon eggshells supplied by R. W. Fyfe, J. H. Enderson, C. White, R. Storer, E. R. Blake, S. G. Sealy, and P. Van Stamm.

five orders is illustrated in Figure 3. This is the response theoretically predicted from the derivation of "relative BBS." The response to intraspecific variation in weight per unit area in fresh, intact common tern eggs and eggshells of the American merlin, *Falco columbarius*, was also determined (Figure 4). Clearly, BBS is sensitive to inter- and intraspecific variation in cation content and thickness of eggshells.

The term "thickness" as used in recent literature refers to both the actual measurement of shell thickness and to measurements of shell weight. As thickness can be independent of weight and does not reflect chemical and structural variation, this usage is clearly in error. I have chosen to use the term "shell quality" to refer to *any* of a number of parameters including thickness, thickness index (relative weight), relative BBS, chemical content, structure, and porosity.

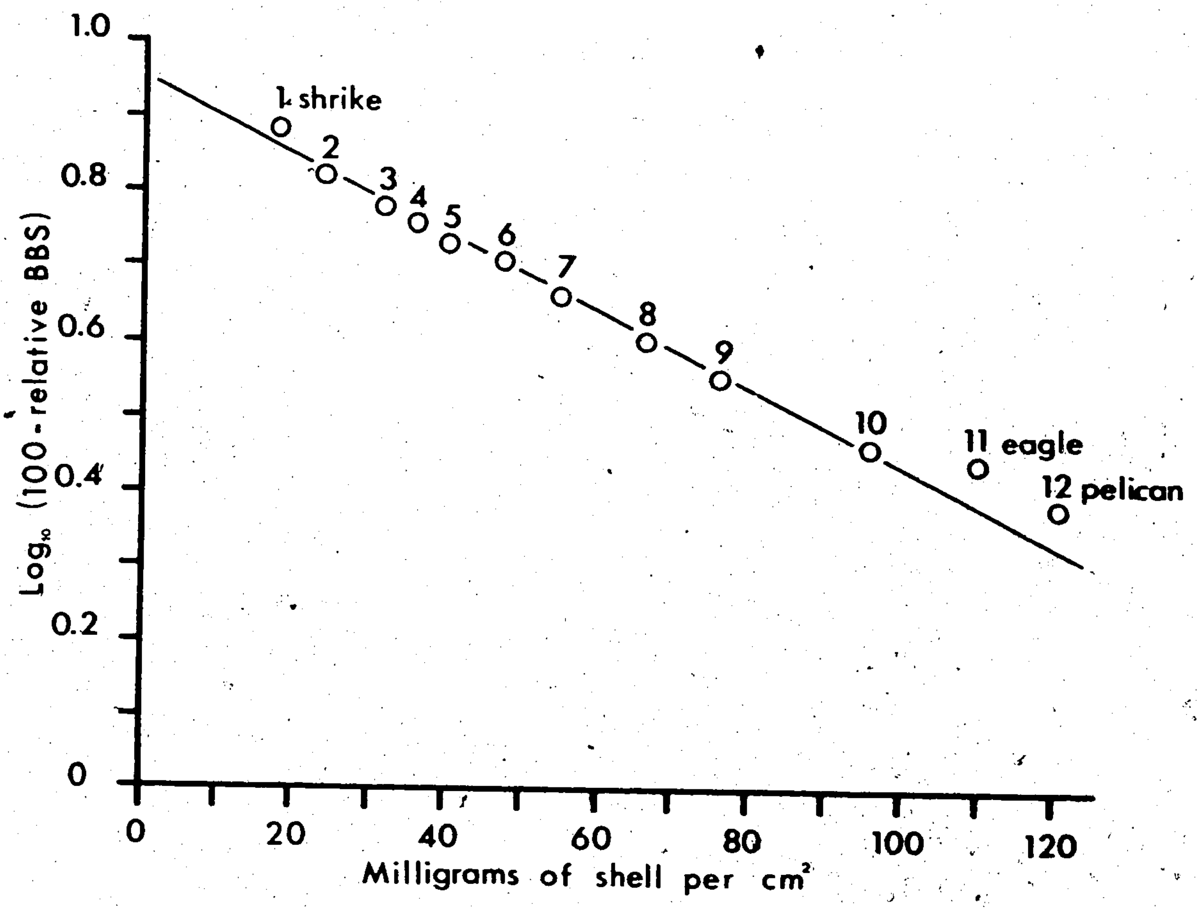


Figure 3. The response of the BBS device to interspecific differences in eggshells of (1) loggerhead shrike, (2) belted kingfisher, (3) common tern, (4) American kestrel, (5) sharp-shinned hawk, (6) American merlin, (7) marsh hawk, (8) peregrine falcon, (9) goshawk, (10) ferruginous hawk, (11) golden eagle, and (12) white pelican. The shrike, eagle, and pelican eggs were of sizes such that they could not be positioned properly hence altering the geometry of the device and resulting in their deviation in linearity. Values plotted are means ($n = 6$ to 10 except for eagle where $n = 2$). Eggs measured are in the collections of the National Museum of Natural Sciences, Ottawa, and the Royal Ontario Museum, Toronto.

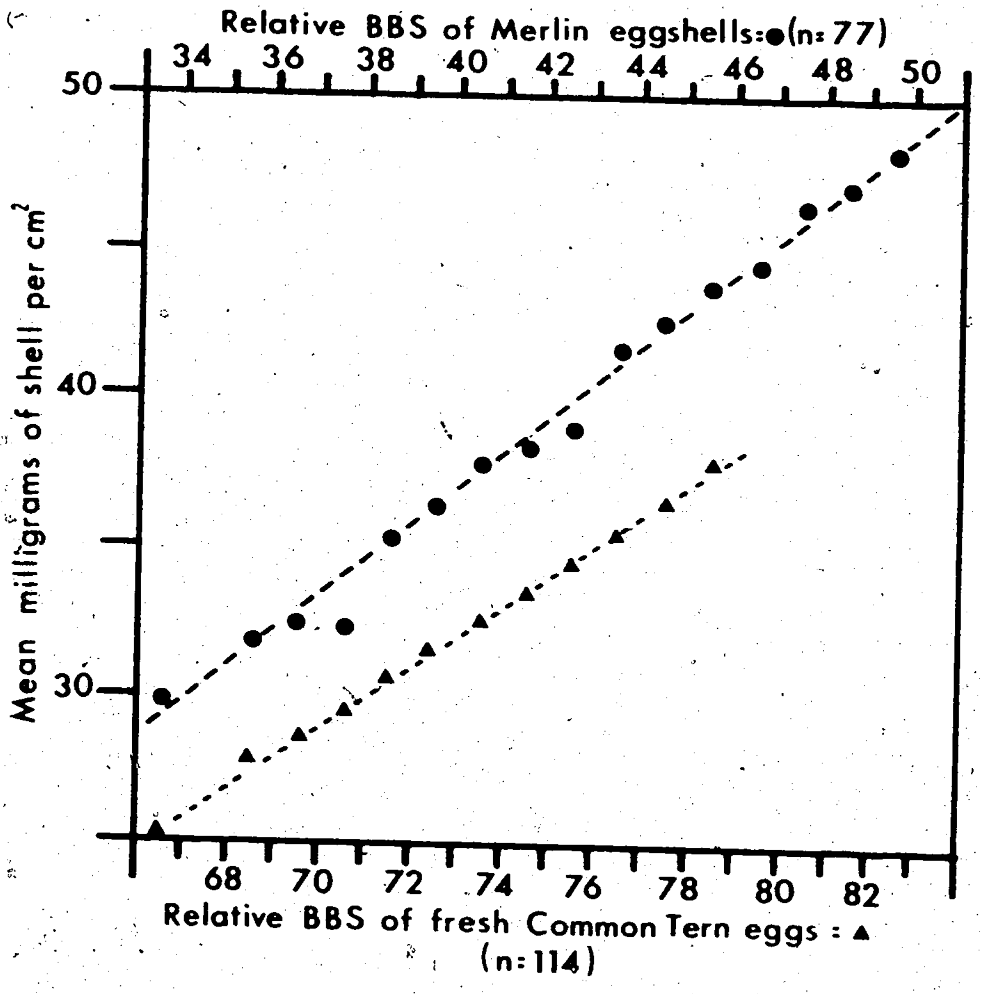


Figure 4. The response of the BBS device to intraspecific variation in eggs of the common tern and eggshells of the American merlin. Values plotted are means for 5 to 10 tern eggs and 1 to 10 merlin eggshells. Merlin eggshells include those in Table 1, plus those in collections of National Museum of Natural Sciences, Ottawa, and the Royal Ontario Museum, Toronto.

RESULTS AND DISCUSSION

NESTING SUCCESS OF THE BUFFALO LAKE TERN COLONY, 1972

A total of 473 eggs were laid in 226 nesting attempts, resulting in a mean clutch size of 2.1 eggs. Of these eggs, 288 (61 percent) hatched. At least 133 of these 288 chicks (46 percent) survived for 14 days. Hence, minimal production in terms of 14-day old chicks was 0.28 per egg laid, and 0.59 per nesting attempt. Eighty-three percent of chick mortality occurred within the first 14 days and hence the figure of 0.28 chick per egg laid closely approximates nesting success.

There are two approaches to assessing nesting success. One is to make comparisons to that theoretically required to maintain a stable population. Alternatively, I have chosen to view it in terms of comparison with other populations of the same species. Langham (1968) and Nisbet (pers. comm.) have made intensive studies, using similar methods, of colonies on Coquet Island, Northumberland, and coastal Massachusetts, respectively. Their findings are presented here as arbitrary standards with which to measure the relative nesting success of the Buffalo Lake terns in 1972 (Table 2). The reproductive effort of the Buffalo Lake terns in 1972 was appreciably less successful than these arbitrary standards. The decreased success was largely the result of egg disappearance, egg damage, and embryonic failure.

TABLE 2

FATE OF EGGS FROM THREE POPULATIONS OF COMMON TERNS, EXPRESSED AS A PERCENTAGE OF TOTAL LAID

	Location of colony		
	Buffalo Lake Alberta 1972[a]	Coquet Island Northumberland 1965[b]	Bird Island Massachusetts 1971[c]
	n = 456	n = 580	n = 200+
Hatched	61**	88	94
Disappeared	5**	0	0
Cracked or dented	4*	1.5	0
Embryo failed	21**	3	2
Abandoned	6	5	3
Fledged young	28**	52	71

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

*Significantly different from Coquet Island ($P < 0.05$).

**Significantly different from Coquet Island ($P < 0.001$).

SHELL QUALITY (BBS) AND ITS RELATIONSHIP TO NESTING SUCCESS IN THE BUFFALO LAKE COLONY IN 1972

Measurements of 456 fresh eggs laid in the Buffalo Lake colony in 1972 revealed a range of relative BBS values of 66.3 to 79.5 percent. A total of 416 of these eggs was used for statistical analysis. Eggs were placed in one of seven classes of equal width spanning the range of BBS values from 70.0 to 76.9 percent. Eggs were also subdivided on the basis of their laying sequence within the clutch (i.e., *A*-, *B*-, and *C*-eggs). The resulting classes contained 6 to 110 eggs. Classes containing less than seven eggs were omitted from the analysis. The various components of nesting success were calculated for each of the BBS classes. The values obtained for each component were ranked. The relationship of BBS to nesting success was determined by comparing the ranks of the components to the rank of the BBS class using Spearman's Coefficient of Rank Correlation (r_S). The results of these calculations are summarized in Table 3. Shell quality, egg weight, and lipid content decrease in successive eggs of the clutch in the common tern (Dunn, 1972a). In the Buffalo Lake colony, no significant difference in mean shell quality (BBS) existed between *A*- and *B*-eggs, but *C*-eggs differed significantly from *A*-eggs. The weight of *A*-, *B*-, and *C*-eggs were all significantly different from each other. Hence, any toxicant-induced variability in shell quality is superimposed upon natural individual and intraclutch variability. This natural variability may mask any correlations resulting from the effects of toxicant-induced variability if eggs are considered independent of their laying sequence. *A*-eggs are present in all clutches, have the highest shell quality, and are the heaviest in any

TABLE 3

CORRELATION BETWEEN RELATIVE BBS CLASSES AND COMPONENTS OF NESTING SUCCESS OF BUFFALO LAKE TERNS IN 1972 BASED ON OUTCOME OF 416 EGGS OF KNOWN SHELL QUALITY AND LAYING SEQUENCE

Percentage of	*A* eggs (n=195, c=6)		*B+C* eggs (n=209, c=6)		*A+B+C* eggs (N=416, c=7)	
	Range	r_s	Range	r_s	Range	r_s
Egg defects	0- 38	-0.543	0- 31	-0.443	0- 33	-0.750*
Eggs laid which disappeared	0- 33	-0.900*	0- 8	-0.371	0- 16	-0.857*
Incubated embryos which hatched	60-100	+0.943**	92-100	+0.486	75-100	+0.955**
Eggs laid which hatched	33- 83	+0.943**	53- 81	+0.086	54- 83	+0.857*
Chicks hatched surviving 14 days	43- 72	+0.029	31- 50	+0.771	40- 63	+0.107
Eggs laid producing 14-day chicks	17- 54	+0.829*	20- 36	+0.549	17- 47	+0.179

(n = number of eggs in sample, c = number of BBS classes represented)

Range = range of values for component of reproductive success encountered in BBS classes.

r_s = Spearman's Coefficient of Rank Correlation. A positive value indicates that the correlation between relative BBS and the component of reproductive success is direct, while a negative value is indicative of an inverse relationship.

Egg defects = failure resulting from cracking or denting, infertility, embryonic death, or microbial decomposition.

*P < 0.05; **P < 0.01.

clutch, and as such are the least biased group for statistical analysis. Clearly hatching success and its components--egg quality, egg disappearance, and embryo survival--are correlated with shell quality. Chick survival was not correlated with shell quality although it approached significance in *B*- and *C*- eggs. These relationships were further tested by comparing the ~~mean~~ BBS value for eggs which hatched with the means for the various failure categories. Eggs which hatched had significantly higher shell quality than those which were dented, cracked, or which disappeared, or in which the embryo died ($P < 0.01$, t-test). Dented shells had the lowest mean shell quality of any group tested.

Dented shells were significantly thinner (12 percent) than a random sample in the Chip Lake colony (Switzer *et al.*, in press). Eggshells of the common egret, *Camerodius albus*, and the great blue heron, *Ardea herodias*, which were broken during incubation were thinner than those from eggs which hatched (Faber *et al.*, 1972). Prairie falcon, *Falco mexicanus*, clutches whose representative thickness index exceeded 1.75 fledged 2.6 young per pair whereas those whose representative thickness index was less than 1.45 fledged 0.3 young per pair (Enderson and Berger, 1970). Broken and deserted eggs of the Cooper's hawk, *Accipiter cooperii*, had shells 15.7 and 17.8 percent thinner than the pre-DDT mean (Snyder *et al.*, 1973).

RELATIONSHIP OF SHELL QUALITY (BBS) TO PARENTAL BEHAVIOR IN THE BUFFALO LAKE COLONY IN 1972

The causes of hatching failure may be subdivided into those which are related to behavior (disappearance, abandonment, pecking, or eating), and those which are the result of a defect in the egg itself (infertility, embryonic death, cracking, denting, rotting). Chi-square analysis, grouping eggs into three divisions containing those with BBS values of less than 72, 72 to 74, and greater than 74 percent, indicate that the distribution of eggs which failed as a result of defects in the egg differed significantly from that of eggs whose failure was related to behavior, and from that of eggs which hatched ($P < 0.005$) The distribution of eggs whose failure was related to behavior did not differ from that of eggs that hatched. Egg disappearance was negatively correlated with shell quality. Failure due to abandonment was positively correlated with the mean date of clutch initiation ($r_s = +0.76$, $P < 0.05$). Failure resulting from pecking was not correlated with shell quality.

No difference was detected in the tendency to retrieve an egg from the nest rim between parents whose eggs had high BBS values and those whose shells had low BBS values. This indicates that no differences in the intensity of incubation instinct accompanied variation in eggshell quality. Ospreys, *Pandion haliaetus*, producing thin-shelled eggs and having a history of low reproductive success raised chicks successfully when given "foster" eggs produced by pairs with a history of high reproductive success (P. Spitzer, pers. comm.). Enderson *et al.* (1973) detected no abnormalities in films of parental behavior of unsuccessful tundra peregrines, *Falco peregrinus*, when compared to those of successful pairs.

RELATIONSHIP OF SHELL QUALITY (BBS) TO CHICK BEHAVIOR IN THE BUFFALO LAKE COLONY IN 1972

Seventeen chicks from eggs with BBS values in excess of 74 percent were tested on the visual cliff. Thirteen (93 percent) of the 14 chicks (82 percent) which reacted to the visual situation chose the visually shallow side. In contrast, of 14 chicks from eggs with BBS values of less than 72 percent, only four (66 percent) of the six (29 percent) which reacted chose the visually shallow side. The differences in the proportion of chicks reacting and in the proportion of chicks tested which responded favorably are statistically significant ($P < 0.10$ and $P < 0.025$ respectively, Chi-square). This behavioral difference was independent of fresh egg weight, and the weight or age of the chick at testing. Avoidance of the cliff edge is indicative of depth perception. Thus, there appeared to be a significant difference in the depth perception in chicks from eggs of differing shell quality in the Buffalo Lake colony in 1972. Nisbet (pers. comm.) tested tern chicks in the Massachusetts colonies in 1973, using the identical procedure, and found 93 percent of those which responded chose the shallow side. The majority of the terns he tested reacted to the visual stimuli.

An animal's response to the lack of visual support is unlearned (Walk and Gibson, 1961). In the gulls, *Larus argentatus* and *L. artricilla*, a negative edge response was detected at 24 hours of age or less (Emlen, 1963; Hailman, 1968). 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 (Quine and Cullen, 1964). Thus, depth or distance perception must have survival value to the tern chicks.

EGG DISAPPEARANCE

Thirteen percent of eggs which failed in the Buffalo Lake colony in 1972 disappeared. Gulls were repeatedly driven from the vicinity of the tern colony and several were killed by the terns during the course of this study. The few eggs which were destroyed by gulls were located in peripheral nests. Dump eggs (5.6 percent of all eggs laid in the colony) 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 ($P < 0.025$). Random disappearance would be expected if disturbance was the cause. Forty-eight percent disappeared during the last week of incubation and another 36 percent after they had been incubated for at least the modal incubation period of 21 days. This distribution is very similar to that of eggs which were broken. Seventy-two percent of broken eggs were rotten and the majority of rotten eggs were cracked.

In a recent study of black ducks, *Anas rubripes*, fed DDE, 42 percent of the eggs disappeared from the nests during incubation (Longcore and Samson, 1973). All of these eggs had been cracked before disappearing. The eggs were removed by the hen and deposited in the water trough. Two species of anatids nesting in the wild have been observed to remove eggs with cracked shells placed in their nests and to deposit them in nearby water bodies (Sowls, 1955). Hence, a mechanism for detection of cracked eggs exists in the anatids at least, and such eggs are usually removed from the nest. It is likely, then, that the high rate of egg disappearance from the nests of the DDE-dosed ducks was a direct result of the fact that 58 percent of the eggs laid cracked. Newton (1973) presents strong circumstantial evidence that egg eating and disappearance in recent nestings of the sparrowhawk, *Accipiter nisus*, followed initial damage to the egg. The incidence of egg disappearance in the Buffalo Lake terns in 1972 was very similar to that of cracks and other damage. I suggest that egg disappearance and breakage in the Buffalo Lake colony were largely the result of parental detection of egg failure, possibly by visual detection of cracks, olfaction in the case of putrifaction, or audition in instances of late embryonic or pipping deaths. I further suggest that these faulty eggs were eaten, thus conserving energy as small fragments of shell and drops of egg contents were often found in the nest scrape.

EMBRYONIC FAILURE

Embryonic failure was responsible for at least 53 percent of the eggs that failed to hatch (21 percent of those laid). Sixty-one percent of these eggs were addled, 17 percent died during the first week, 4 percent during the second week, and 18 percent during the third week including 14 percent which died during pipping. This distribution differs significantly from a constant rate ($P < 0.005$), which might be expected if disturbance was the cause. It is unlikely that embryonic nutrition is at fault as the majority of mortality occurred very early in embryogenesis. The proportion of addled eggs to deaths of visible embryos is significantly higher than that observed in eggs of *Sterna paradisaea* nesting on Machias Seal Island in 1937 (Pettingill, 1939). Embryonic failure occurred in less than 5 percent of 868 eggs produced in recent nestings of a colony of caspian terns, *Hydroprogne caspia*, in Finland

(Soikkeli, 1973). The age distribution of embryonic deaths observed in Buffalo Lake terns in 1972 does not differ significantly from that observed in recent nestings of the sparrowhawk (Newton, 1973). Hence, the rate of embryonic failure in the Buffalo Lake colony is significantly higher than that observed in other tern species and resembles that of a declining population of sparrowhawks heavily contaminated with chlorinated hydrocarbons.

MICROBIAL DECOMPOSITION OR ROTTING

The contents of 50 percent of the eggs which failed as a result of egg defects showed obvious signs of microbial decomposition (bad odour, discoloration, and abnormal consistency). Cracking and rotting were not independent ($P < 0.005$). Cracking may be the result of production of gases within the egg by microorganisms. The micro-organisms may be present in the oviduct when the egg is formed or they may gain access to the egg contents through abnormal pores, defective cuticle, or cracks in the shell. Bacteria of five genera were isolated from the egg contents and the Buffalo Lake environment (Table 4). All were Gram-negative coliflora. *Klebsiella* sp. and *E. coli* would appear to be the microorganisms most frequently associated with egg failure. The high incidence of *Klebsiella* sp. in fresh eggs may indicate that it is present in the oviduct. The incidence of other species associated with egg failure parallels that in the environment. *Proteus* sp. and *Streptococcus faecalis* were isolated from embryonated eggs of kestrels which failed to hatch in a captive breeding project (Porter and Wiemeyer, 1970). However, nest box sanitation did not appear to be important in

TABLE 4

INCIDENCE OF MICROORGANISMS IN THE ENVIRONMENT AND EGG CONTENTS OF BUFFALO LAKE TERNS

Microorganism	Incidence in percent		
	Environment (12)	Fresh eggs (5)	Failures (27)
Klebsiella sp.*	17	60	56
Aerobacter sp.	8	0	18
Escherichia coli	58	0	41
Proteus sp.	33	0	22
Aeromonas sp.	8	0	7

( ) = number of items sampled.

*Organism most like *K. edwardsii* var. *edwardsii*.

causing embryonic mortality in one wild population of this species (Heintzelman, 1971).

CHARACTERISTICS OF TERN EGGSHELLS WHICH ACCOMPANIED EGG FAILURE

Weight, Measurements, and BBS

Eighty-seven eggs collected in Alberta, Saskatchewan, and Manitoba prior to 1945 were weighed, L and B measured, and relative BBS values determined. This sample contained eggs collected from nine colonies, in eight years spanning the period 1894 to 1940, and as such should be representative of the geographic and temporal variability in shells of this species on the Canadian Prairies prior to the advent of chlorinated hydrocarbons. Thirteen clutches containing a total of 35 eggs collected at Crane Lake, Saskatchewan, in 1894 provide a measure of intracolonial variability. The BBS values for the empty shells were converted to their intact equivalent by the equation:

$$\text{Intact BBS} = 20.73 + 1.43 \text{ (BBS of empty shell)}$$

This equation was derived by regression analysis of the intact and empty BBS values for 100 tern eggs collected in 1972.

The BBS distribution of 456 eggs produced in the Buffalo Lake colony in 1972 was significantly different from both pre-DDT samples ($P < 0.005$) (Figure 5). The Buffalo Lake terns produced a larger proportion of eggshells of lower quality and there is a downward shift in the modal class.

There was no significant difference in the mean thickness index of 87 eggshells collected in 1972 when compared to the pre-DDT sample

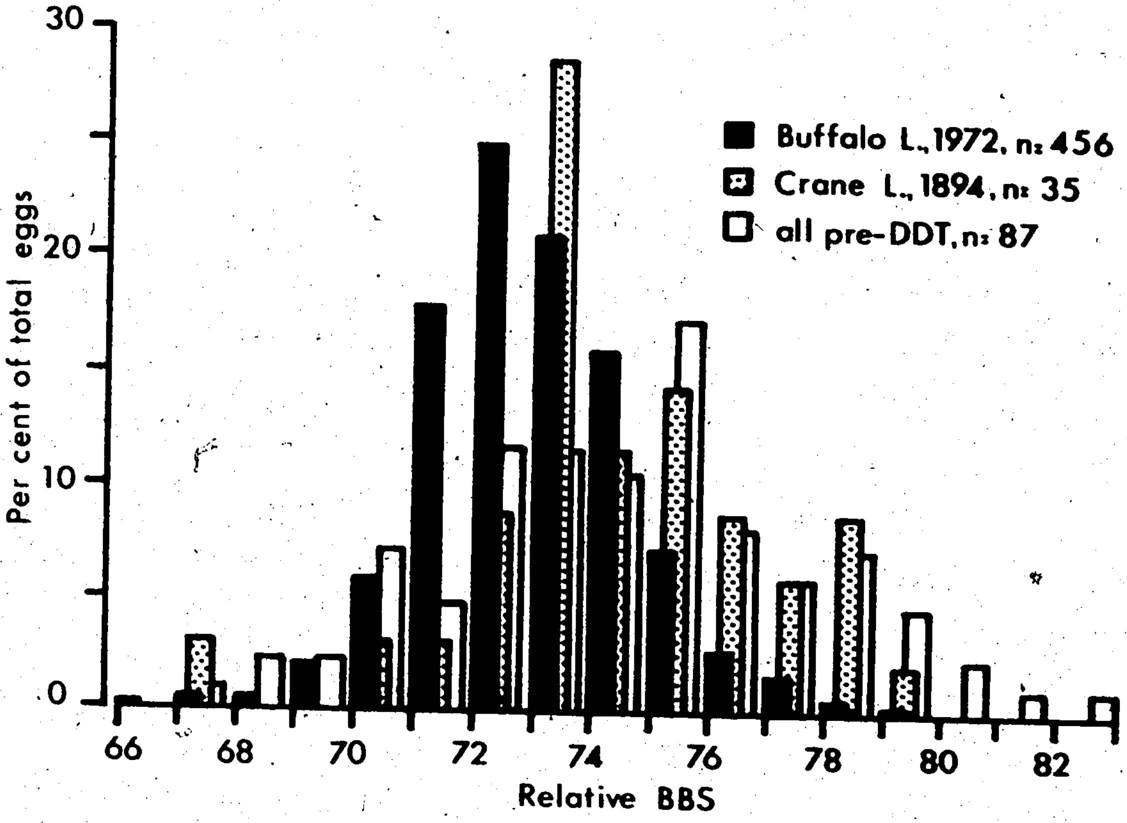


Figure 5. BBS distributions of common tern eggs collected at Buffalo Lake in 1972; Crane Lake, Saskatchewan in 1894; and Alberta, Saskatchewan, and Manitoba prior to the advent of DDT. This figure illustrates both temporal and geographic variability in shell quality. BBS of empty shells was converted to the equivalent of intact eggs mathematically. Pre-DDT eggshells are in collections of National Museum of Natural Sciences, Ottawa; Royal Ontario Museum; and the University of California Museum of Vertebrate Zoology.

(0.918 *vs.* 0.920). However, the mean thickness index of dented shells was 12 percent less than the 1972 mean for the colony as a whole, and 15 percent less than the pre-DDT mean ($P < 0.05$). The mean shell weight of eggs collected in 1972 (1.13 g) was 5 percent less than that of the pre-DDT sample. The mean shell weight of eggs collected from the Chip Lake colony in 1970 (Switzer *et al.*, in press) was 15 percent less than that of my pre-DDT sample ($P < 0.01$).

Chemical and Physical Characteristics

It is generally accepted that avian eggshell is primarily composed of calcium carbonate in the form of calcite (Heyn, 1963; Simkiss and Taylor, 1971). However, a large number of elements are known to be present in trace quantities. Some of these elements exist as impurities while others such as phosphorus and magnesium are present in the form of salts.

The chemical and physical characteristics of 35 shells collected in the Buffalo Lake colony in 1972, and of three collected prior to the introduction of DDT, are summarized in Table 5. The only consistent difference between the chemical composition of shells of eggs which failed and viable or pre-DDT eggs was a marked increase in their phosphorus content. It would appear that PO_3^- groups substituted for CO_3^{--} ions in the crystal lattice. These shells have a molar concentration of phosphorus which is three times that of viable and pre-DDT eggs. A three-fold increase in the phosphorus content of the shell is sufficient to bind an equal concentration of calcium ions per unit weight in the form of tricalcium phosphate ($Ca_3(PO_4)_2$) or hydroxyapatite ($Ca_5(PO_4)_3OH$) as calcium carbonate ($CaCO_3$).

The substitution of PO_3^- for CO_3^{--} ions in the calcite lattice has

TABLE 5

VARIATION IN PHYSICAL AND CHEMICAL CHARACTERISTICS BETWEEN THE SHELLS OF PRE-DDT, VIABLE, AND VARIOUS FAILURE CLASSES OF COMMON TERN EGGS

	Pre-DDT (3)	Viable (12)	Early deaths (5)	Addled* (13)	Dents (5)
Thickness, μ	200	197	205	205	175
Ca, mg/cm^2	13.18	10.54	10.55	11.64	10.80
Percent Ca	39.08	34.68	32.42	34.79	37.78
Percent Mg	17.99	15.44	16.42	13.53	21.01
Percent P	0.27	0.24	0.69	0.92	0.63
Ca:P, molar	111.1	111.1	34.5	29.4	45.5
Mg:Ca, molar	0.76	0.73	0.83	0.64	0.92

(Sample size)

*No visible embryo

Means underlined by the same line are not significantly different ($P > 0.05$).

the following effects: (1) it distorts the calcite crystal because the foreign metaphosphate ions fit the crystal in two dimensions but not in the third, and (2) it removes the electrostatic potential for the absorption of another layer of calcium ions, stopping crystal growth (Simkiss, 1964). Hence, this substitution has potential structural effects.

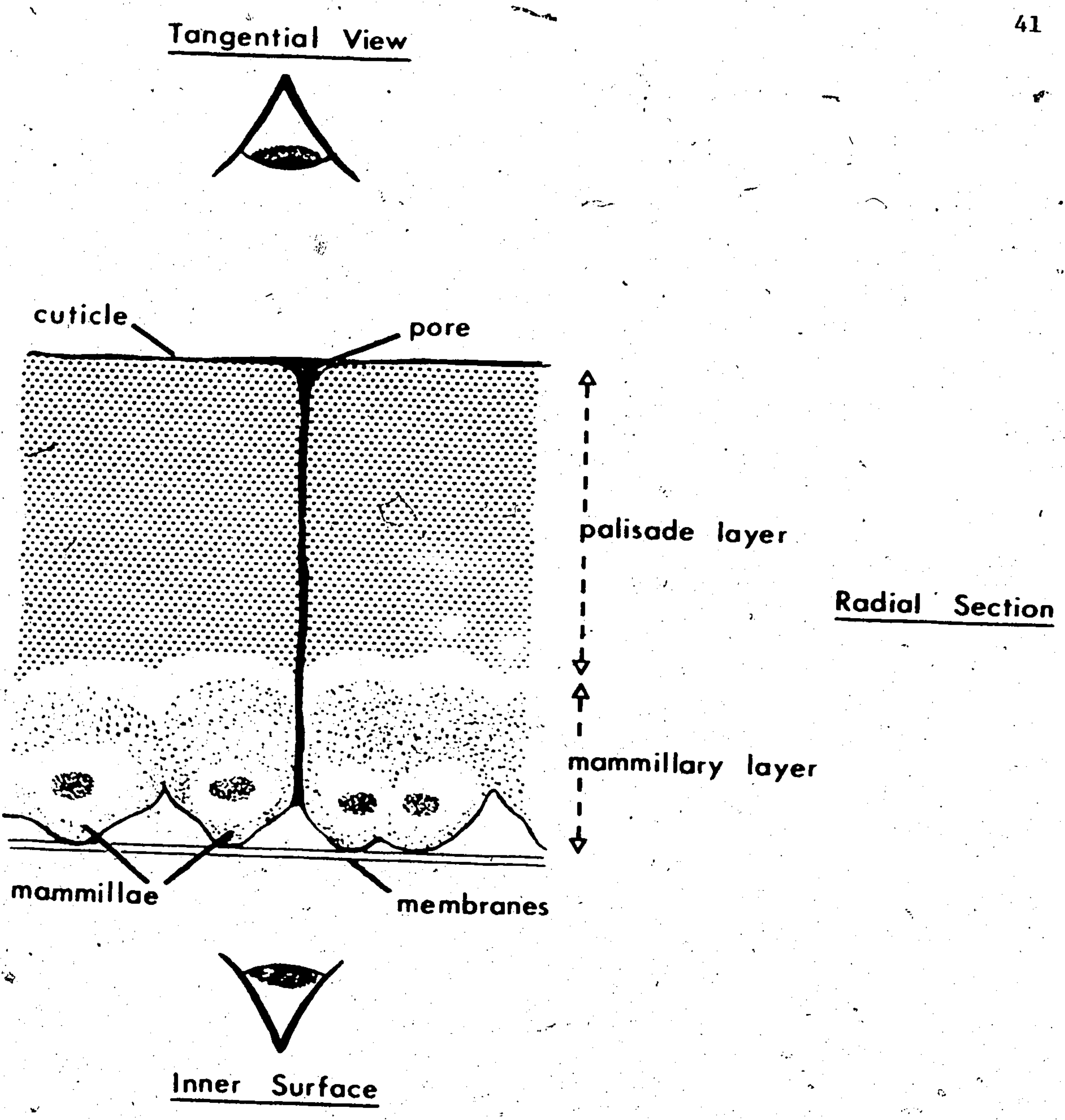
Dented shells were significantly thinner and weighed 13.5 percent less per unit area than shells of pre-DDT or viable eggs but contained the same weight of calcium. These shells had an altered phosphorus content. This increased phosphorus content may be responsible for their reduced strength, and in part, for their reduced thickness as a result of decreased and abnormal crystal growth. In addition, dented shells had a higher Mg:Ca ratio than other shells. An increase in the Mg:Ca ratio may increase hardness and brittleness of eggshells (Brooks and Hale, 1955).

Structural Characteristics

Normal eggshell structure. The eggshell lies immediately external to the shell membranes. The inner or mammillary layer of the shell consists of roughly hemispherical knobs in the inner end of which there is an organic core. These organic cores represent the initial nucleation sites which initiate and orientate crystal growth. The fibers of the outermost shell membrane pass through these cores and firmly attach the membrane to the shell. The diameter of the mammillae increases with distance from the shell membrane and they ultimately fuse together. The mammillary layer is continuous with the spongy or palisade layer which comprises most of the shell thickness. The spongy layer has as its nucleation sites the mammillary bodies and thus the crystals are arranged in bundles corresponding to the maximum diameter of the mammillary bodies and junctions between bundles are plainly visible in tangential sections of the spongy layer. Channels arise from some of the spaces between mammillae and pass through the spongy layer, opening on the surface of the egg in small depressions. These pores make gaseous exchange between the embryo and the atmosphere possible. The outer surface of the egg is covered with an organic cuticle. This basic structure is schematically represented in Figure 6.

Structure of eggshells collected in 1972. The differences in microscopic structure between eggs which hatched and those which failed to hatch are presented in Figures 7 and 8. During the latter part of the incubation period, calcium is withdrawn from the shell. This occurs at the mammillary cores. Hence the eroded central depressions in the center of the bases of mammillae in Figure 7a. This decalcification does not alter the number of mammillae, their size, or distribution, nor does it alter the number of pores. Hence, comparisons made between eggs which hatched or contained viable embryos and eggs which failed to hatch are valid.

In general terms, egg failure was accompanied by less organized structure. There were fewer mammillary bodies per representative field photographed (45.9 *vs.* 58.5, $n = 4$ and 10, $P < 0.05$) and this decrease in number was accompanied by marked variation in size and shape. There were 44 percent fewer pores on the inner surface. This decrease in pore numbers approached statistical significance ($P < 0.10$). The mammillary bodies, which normally fit tightly together, had irregularly shaped



re 6. Diagrammatic representation of a radial section of an eggshell showing the major structural features and orientation of views photographed with the scanning electron microscope and illustrated in Figures 7, 8, 9, and 12.

Figure 7. Structural characteristics of shells of viable and non-viable common tern eggs collected in the Buffalo Lake colony in 1972 as revealed by the scanning electron microscope.

Inner surface with membranes removed

A. Viable (168X). Densely packed, evenly distributed mammillae. Prominent pores (*arrows*).

B & D. Non-viable (168X). Less densely packed, unevenly distributed, larger mammillae. Fewer and less prominent pores (*arrows*).

Tangential view with cuticle removed

C. Viable (1690X). A normal pore at junction of four mammillae.

E. Viable (1680X). Close fitting, regular shaped junctions between mammillae.

F. Non-viable (840X). Junctions between mammillae are irregular in shape and widely separated with prominent "inter-mammillary plugs" of material showing varied crystalline orientation.

Figure 8. Structural characteristics of shells of viable and non-viable common tern eggs collected in the Buffalo Lake colony in 1972 as revealed by the scanning electron microscope.

Radial view of broken edge

A. Viable (770X). Tightly packed mammillae with even, spongy appearance. Denser outer layer and cuticle present.

B. Non-viable (420X). Mammillae less densely packed. Disorganized palisade layer. Absence of dense outer region of palisade layer. Cuticle absent.

Untreated outer surface

C. Viable (168X). Cuticle present with prominent pore plugs (*arrow*).

D. Non-viable (168X). Cuticle absent and no pores or pore plugs visible.

gaps between them, composed of crystals of varying orientation. These "intermammillary plugs" appeared to replace pores in some cases. The spongy layer was less organized and contained more, and larger, globular inclusions and in many cases failed to exhibit the normal increased density near the surface (Figure 8b). In cases where the shell was thin, the spongy layer was markedly reduced. The cuticle appeared abnormal or absent in many non-viable eggs.

An egg which was collapsed on laying showed little development of the palisade layer, and consisted primarily of mammillary bodies which were barely fused together (Figure 9a). The shell was pigmented and thus was probably not extruded prematurely. The contents of this egg were markedly desiccated three days after laying. Another egg, the apex of which was dented inwards upon laying, was also deficient in the palisade layer but complete fusion was attained (Figure 9b). The fusion was attained rather close to the surface and thus this shell was inherently weak. The number and distribution of mammillary bodies were normal as was the external surface. Several eggs collected in 1972 showed globular projections on the outer surface (Figures 9c and 9d), similar to those associated with chlorinated hydrocarbons (Erben and Krampitz, 1971; McFarland *et al.*, 1971; and Peakall *et al.*, in press).

The number and size of mammillae are important to shell strength as measured by resistance to deformation. A reduction in numbers and increase in the size of mammillae are clearly accompanied by a decrease in strength of eggs of domestic fowl (Simons, 1971). Irregular distribution of mammillae and an increase in the number of globular inclusions in the spongy layer are also accompanied by a decrease in strength (Robinson and King, 1970). It is thus apparent that the changes

Figure 9. Structural abnormalities in shells of common tern eggs collected in the Buffalo Lake colony as revealed by the scanning electron microscope.

A. Oblique view of outer surface (1680X) of a shell which collapsed on laying. Note prominent fissures between mammillae (*arrow*) and lack of palisade layer.

B. Oblique view of radial surface (840X) of a shell whose apex dented inward upon laying. Note poorly developed palisade layer and disorganized structure. Some mammillae do not fuse until very close to the surface (*arrow*).

C & D. Views of the outer surface (1680X) of two non-viable eggs. Note the large number of globular projections which have been associated with chlorinated hydrocarbon residues by other authors.

in shell structure which accompanied failure to hatch in the Buffalo Lake colony in 1972 would reduce strength. A decrease in pore numbers without a marked increase in pore size can only lead to decreased gaseous exchange. The abnormality in, or absence of, cuticle reduces or eliminates the major barrier to microbial penetration. These changes are thus all of a deleterious nature.

Evaporative Water Loss and Gaseous Exchange

Normal embryonic development requires gaseous exchange between the embryo and its external environment. This is accomplished by simple diffusion through the pores in the shell. These pores restrict the oxygen supply and determine the CO_2 level of the embryonic blood as well as water loss during incubation. As the total pore area is established at the time the egg is formed, any abnormality in pore area will have a deleterious effect upon embryonic survival (Wangensteen and Rahn, 1970/71).

The continuous water loss by the egg depends on the relative humidity of the environment and the permeability of the eggshell to water vapor which is determined by the pore area (Wangensteen and Rahn, 1970/71). The measurement of evaporative water loss during normal incubation can be used as a measure of pore area and gaseous exchange. The mean evaporative water loss of 26 eggs which were incubated but failed to hatch in the Buffalo Lake colony was 2.7 mg/cm^2 per day. This was significantly ($P < 0.001$) less than the mean of 4.4 mg/cm^2 per day attained by 15 eggs which hatched. This 38 percent difference implies a marked decrease in total pore area. Indeed, in a limited sample examined with the scanning electron microscope, a 44 percent decrease in total pores was observed ($P < 0.10$). Drent (1970) found a linear relationship between daily weight loss during natural incubation and the initial egg weight based on data presented in 57 studies of 46 species of birds ranging in size from the house wren, *Troglodytes aedon*, to the sandhill crane, *Grus canadensis*. The evaporation per unit surface area was 4.5 mg/cm^2 per day despite the range in egg weight of 1.5 to 170 g. In light of this interspecific consistency, it is very unlikely that such marked intraspecific differences as those found in the Buffalo Lake terns can be considered as normal.

Reduced atmospheric oxygen concentration accompanied by increased carbon dioxide concentrations produced marked decreases in hatchability of chicken eggs (Taylor *et al.*, 1956). This mortality is marked even at 48 hours. A reduction of the pore area of chicken eggs by 25 percent reduced the hatchability by 38 percent (Tazawa, 1972). Hence, it is likely that the reduced pore area and resulting reductions in gaseous exchange are in part responsible for the reduced hatchability observed in the Buffalo Lake colony in 1972.

CHLORINATED HYDROCARBONS AND THEIR POSSIBLE ROLE IN REDUCING SHELL QUALITY OF BUFFALO LAKE TERNS

A total of 44 eggs collected in the Buffalo Lake colony in 1972 was subjected to analysis for chlorinated hydrocarbon residues. This sample represents about 10 percent of the eggs laid and is biased towards eggs which failed to hatch or which were obviously abnormal. As in all studies to date, no residue values are available for eggs which hatched. DDE and PCB's were present in all pooled samples and individual eggs examined. Dieldrin was virtually absent. The mean *p,p'*-DDE content was

3.80 ppm (wet weight basis). A range of 0.50 to 11.08 ppm was encountered in 13 individual eggs. PCB's were not quantified. 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 these terns while present at Buffalo Lake, contained 0.02 ppm *p,p'*-DDE. PCB's and Dieldrin were absent. It is apparent that the body burden of DDE, which is reflected in the eggs of Buffalo Lake terns, is not accumulated on the breeding grounds. Recovery of eight terns banded in Alberta colonies indicates that they winter and spend the first two 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 chlorinated hydrocarbons (Burnett, 1971; Duke and Wilson, 1971; and Risebrough *et al.*, 1967).

In contrast, five eggs collected from the successful Coquet Island colony in 1965 contained a mean of 0.04 ppm *p,p'*-DDE and food fish contained 0.03 to 0.08 ppm (Robinson *et al.*, 1967). Hence 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 two colonies were similar.

The 13 individual eggs contained a mean DDE content of 2.25 ppm while seven pooled samples representing a total of 31 eggs contained a mean of 4.50 ppm. The apparent bias towards eggs with lower than average residues in the individual eggs sampled and the small sample size limits the value of regression analysis. DDE was negatively correlated with the thickness index ($r_s = -0.650$, $P < 0.05$) and phosphorus content ($r_s = -0.683$, $P < 0.05$).

A pooled sample of five eggs with shells of very low quality contained 6.77 ppm DDE, approximately twice the mean for all eggs, and the most DDE of seven pools examined. The thickness index of these eggs was 17 percent less than the pre-DDT mean.

Common tern eggs collected at Chip Lake, Alberta, in 1970 had a mean *p,p'*-DDE residue of 4.25 ppm, and a range of 0.13 to 26.17 ppm (Switzer *et al.*, in press). Eggs collected at random from this colony yielded a significant negative correlation between shell thickness and DDE residues ($r_s = -0.521$, $P < 0.01$). Dented eggs contained significantly more DDE (mean = 7.52 ppm) and were 12 percent thinner than randomly collected eggs. These values are very similar to those obtained for the Buffalo Lake colony in 1972.

The low reproductive success of Buffalo Lake terns in 1972 was largely the result of egg disappearance, egg damage, and embryonic failure. It was thought that egg disappearance usually followed embryonic failure. Hence, embryonic failure was thought to account for 66 percent of the eggs which failed to hatch while an additional 10 percent were damaged.

Shell damage in the form of denting occurred only in eggs of low shell quality, and was accompanied by higher-than-average DDE residues. Based on the coefficient of determination (r_s^2), 42 percent of the variability in the thickness index of Buffalo Lake terns can be explained by DDE residues. Switzer *et al.* (in press), using the same criterion, were able to explain 27 percent of the variability in shell thickness of Chip Lake terns on the basis of DDE residues. As eggshell quality is affected by a number of physiological and environmental variables under normal conditions, any variable that accounts for 27 to 42 percent of the variability must be considered as biologically significant.

Embryonic failure was accompanied by decreased evaporative water loss. This decrease was accompanied by a similar decrease in pore numbers, but no consistent change in shell thickness was observed. Peakall *et al.* (in press) induced shell thinning and 15 to 25 percent decreases in evaporative water loss in eggs of American kestrels, ring doves, *Streptopelia risoria*, and white pekin ducks by feeding DDE. These changes were also accompanied by a change in the number of globular inclusions. Erben and Krampitz (1971) observed that the most marked abnormalities in recent brown pelican, *Pelecanus occidentalis*, eggshells were an increase in the number of globular inclusions which decrease the compactness of the shell, and changes in the amino acid composition of the matrix substance. The mammillary cores, which are the nucleation sites of the mammillary bodies, are composed of matrix substance. Alteration of the amino acid content could alter the net charge of the matrix substance which could in turn alter the number and distribution of the mammillary bodies. Common tern eggs which failed had significantly fewer mammillary bodies per unit area, and these differed markedly in their size and shape. The size, number, and distribution of mammillae affect shell strength (Robinson and King, 1970; Simons, 1971). Peakall *et al.* (in press) found that s[illegible] of their DDE-dosed duck eggs had lower breaking strength and a different thickness:strength relation than those of the control group. This may well reflect changes in the size, number, and distribution of mammillae. The matrix composition may determine what "morph" of calcium carbonate is deposited during biomineralization (Watabe and Wilbur, 1960). Dented tern eggshells had increased Mg:Ca ratios. Longcore *et al.* (1970) induced a similar change in the Mg:Ca ratio which was accompanied by a marked increase in cracking in the shells of black ducks by feeding DDE.

Hence, physiological, structural, and chemical changes in eggshells which were observed to accompany embryonic failure in the Buffalo Lake terns have been induced experimentally in several species by feeding DDE, and have been observed in thin eggshells of the brown pelican collected from Anacapa Island. Fifty-eight percent of the variation in the thickness index of recent brown pelican eggs can be explained by their DDE content (Blus *et al.*, 1971).

Few laboratory studies of the effects of DDE-contaminated food on avian reproduction have included detailed analysis of reproductive success which might be used in comparison with field studies. Porter and Weimeyer (1969) fed 0.28 ppm Dieldrin plus 1.4 ppm DDE to captive American kestrels. This dosage resulted in eggshell thinning, increaaed disappearance, increased embryonic death, and decreased hatchability. Longcore *et al.* (1971) fed 3 ppm DDE to penned black ducks and observed shell thinning, increased cracking, a marked increase in early embryonic deaths, decreased hatchability, and decreased duckling survival. Jefferies (1971) fed 4 ppm DDE to Bengalese finches, *Lonchura striata*, and found no shell thinning but decreased embryonation, increased embryonic death, and decreased hatchability and chick survival. Thus, increased egg disappearance, decreased embryonation, increased embryonic mortality, the major causes of reduced reproductive success in the Buffalo Lake tern colony, have all been observed in controlled laboratory studies of three species representing three very distinct orders, fed low dietary levels of DDE. It does not seem unreasonable to conclude that DDE is responsible for the low reproductive success of the Buffalo Lake terns.

The apparent 50 percent reduction in nesting success of Buffalo Lake terns which accompanies mean egg residues of less than 4 DDE and little or no shell thinning is indicative of a very marked sensitivity to DDE. In other species, such decreases in reproductive success are accompanied by residues in excess of 20 ppm and usually a decrease in the thickness index of 15 percent or more. This type of response has not been recorded before.

THE POSSIBLE ROLE OF CHLORINATED HYDROCARBONS IN ALTERING TERN BEHAVIOR

Significantly inferior visual cliff behavior was observed in tern chicks which hatched from eggs with low quality shells. Such eggs contained more DDE than those with high shell quality. Feeding of chlorinated hydrocarbons to penned pheasants, *Phasianus colchicus*, altered the visual cliff performance of their chicks (Baxter *et al.*, 1971; Dalgren and Linder, 1971). Revzin (1966) found that chlorinated hydrocarbons selectively affect 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 depth perception in tern chicks is likely the result of the presence of a toxicant in the egg contents, a toxicant which is also capable of altering shell quality. DDE is such a toxicant.

No differences in chick survival or growth could be associated with their visual cliff performance. This may have been the result of small sample size, the masking of effects by other variables, or simply because the chick is fed by the adult and thus is not an active predator. Survival and growth of *C*-chicks was positively correlated with the feeding performance of the parent male (Nisbet, 1973a). Terns are sight-oriented, plunge-diving predators and as such require good depth perception (Dunn, 1972b; Salt and Willard, 1971). Hence, altered depth 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.

EVIDENCE OF CHANGES IN POPULATION DYNAMICS

Nisbet (1973b) indicates that the breeding success of populations of common terns nesting in Massachusetts has declined markedly since the 1900's. Numbers have declined steadily since 1920 (most markedly since 1950), and now represent about 20 percent of the peak population. The reduction in reproductive success he feels is through reduced chick survival. Nisbet (pers. comm.) feels that a minimal production of 1.1 fledged chicks/pair per year is necessary to maintain stability. Recruitment in the Buffalo Lake and Chip Lake colonies has been much lower than this minimal rate in recent years. However, no population declines have been observed in these inland colonies. However, tern numbers at inland colonies fluctuate markedly and reflect the availability of nesting habitat which varies with water levels. A long-term study of a single colony of marked individuals is necessary to determine the nature of population dynamics.

There has been little banding of common terns in Alberta in the past. Houston's data (1972 and pers. comm.) for Saskatchewan are based on 2,759 banded chicks from two locations and an additional 470 from two others, banded between 1930 and 1969, and thus encompass both the pre- and post-DDE eras. The recovery data he presents indicate that the Saskatchewan population winters in the same general area as the Alberta population. Hence it is likely that they carry a similar residue burden. This is the only species which Houston has banded in Saskatchewan in which the recovery rate since 1950 exceeds that for the 1930's (Figure 10). There has also been a decrease in the age at recovery (Figure 10). Since 1960, no individuals have been recovered at ages greater than 36 months, whereas prior to 1950, the mean age at recovery was 65 months. Such changes may result in reduced recruitment and altered age distribution within the population. Of 727 tern chicks banded by Lewin between 1970 and 1972 at three Alberta colonies, five (0.69 percent) have been recovered to date at locations other than the banding site at ages ranging from one to 15 months. Hence, the recovery rate of terns hatched in Alberta since 1970 is even greater than that of those hatched in the 1960's in Saskatchewan.

COMPARISON OF FACTORS AFFECTING REPRODUCTIVE SUCCESS IN TERNS AND FALCONS

The strongest evidence linking chlorinated hydrocarbons to decreased eggshell quality and accompanying population declines exist for the Falconiformes (Cade *et al.*, 1971; Fyfe *et al.*, 1969; Lincer, 1972; Lockie *et al.*, 1969; and Snyder *et al.*, 1973). Lincer has presented data that experimentally confirm the existence of a cause-and-effect

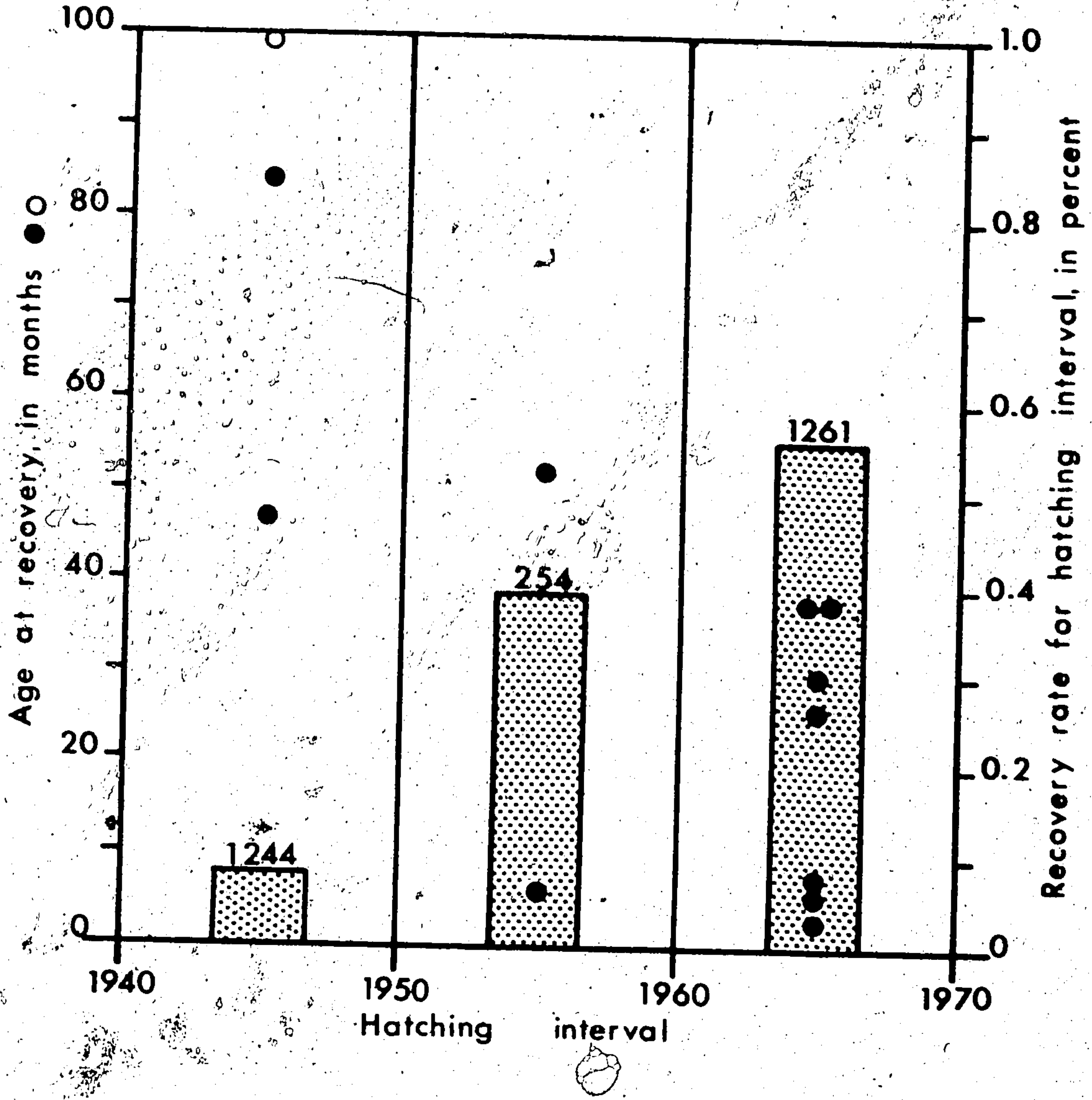


Figure 10. The recovery rate and age at recovery of common terns hatched and banded in Saskatchewan (based on Houston, 1972 and pers. comm.). Recovery rates are calculated for chicks banded at Last Mountain and Redberry Lake. Age at recovery data also include chicks banded at Dore Lake. Open symbol represents individual trapped as a breeding adult. Closed symbols represent injured or dead individuals. Bars indicate recovery rate and numbers above the bars indicate total chicks banded in interval for which recovery rate is shown.

relationship. No studies of changes in eggshell chemistry or structure in raptorial birds have been published. Hence, they were performed as part of this study and are included here for comparison.

Fox (1971) documented that marked changes in shell weight accompanied reduced reproductive success in the American merlin, and that populations had indeed declined. R. Fyfe (pers. comm.) has indicated that changes in thickness index in shells of this species correlate highly with DDE residues in the egg contents. Merlin eggshells collected during the studies of Fox and Fyfe were examined.

The hatching success of the Buffalo Lake terns was 27 percent lower than the Coquet Island population. Embryonic failure accounted for 53 percent of the difference and egg disappearance an additional 23 percent. In the merlins studied by Fox (1971), hatching success was 32 percent lower than that recorded in pre-DDT times. Embryonic failure was responsible for 36 percent of the difference while egg disappearance accounted for the remaining 64 percent. Hence, although the net change in reproductive success of the two species is similar, the mechanism is apparently different. There has been a marked change in the thickness index in the merlin (Figure 11), and thus shell damage is likely to be considerable. Removal or destruction, and thus disappearance, of these eggs is likely. Only a slight decrease in the thickness index has occurred in the terns (Figure 11), and embryonic death, probably through changes in the respiratory properties of the shell, is the major cause of embryonic failure.

The only common chemical differences accompanying decreased shell quality were an increase in both the magnesium content and the Mg:Ca ratio (Table 6). The effect of the latter is to increase the brittleness

TABLE 6

COMPARISON OF SHELL CHARACTERISTICS ACCOMPANYING SHELL THINNING AND REDUCED REPRODUCTIVE SUCCESS IN COMMON TERNS AND AMERICAN MERLIN

Percent change in parameter[a]	Common Tern (n = 17)	American Merlin (n = 26)
Thickness index	-13*	-23**
Calcium per cm^2	none	-21**
Magnesium per cm^2	+13*	+ 4
Phosphorus per cm^2	+24*	variable
Ca:P ratio	-66*	none
Mg:Ca ratio	+20*	+22*

Correlation (r_s) with DDE	Common Tern (n = 9)	American Merlin (n = 15)
BBS	-0.550	-0.660**
Thickness index	-0.650*	-0.670**
Calcium per cm^2	-0.438	-0.573*
Magnesium per cm^2	+0.262	+0.559*
Phosphorus per cm^2	-0.683*	+0.316

[a] Viable *vs.* dented tern eggs; recent *vs.* pre-DDT merlin eggs.

*$P < 0.05$; **$P < 0.01$.

of the shell. In both species, based on a very limited sample, thickness index varied inversely with the DDE content of the egg, reflecting the inverse relationship between residues and calcium carbonate content (Table 6).

The shells of tern eggs which failed do not show similar structural changes to those seen in recent merlin eggshells. In terns, the major changes are related to the size, number, and distribution of the mammillary bodies (Figures 7 and 8). In the merlin, there is some indication that the distribution of mammillae has become less uniform, but changes in size and number were not marked (Figure 12). Pore numbers were markedly reduced in the tern shells but little change was observed in the merlin shells, although pores may be smaller. The most striking change in the merlin shells was in their mammillae. They have narrow, porous bases, and many do not appear to be connected with the membranes. In others, the attachment to the membranes is abnormal. Fusion of the bodies occurs much deeper in the shell, which presumably reduces strength. Differences in crystal size and orientation which contribute to shell texture were also observed. Some of these changes are similar to those induced by MacFarland *et al.* (1971) by feeding large amounts of chlorinated hydrocarbons to Japanese quail, *Coturnix coturnix japonica.*

CONCLUDING DISCUSSION

There is abundant evidence that eggshell thinning has accompanied reduced reproductive success in bird- and fish-eating avifauna. There is also strong evidence implicating chlorinated hydrocarbons, particularly DDE, as the agent responsible for this thinning in accipiters, falcons,

Figure 12. Structural characteristics of pre- and post-DDT American merlin eggshells as revealed by the scanning electron microscope. A, C, and E are pre-DDT shells. B, D, and F are of recent shells.

<u>Radial view of broken edge</u> (336X)

A & B. Recent eggs have abnormal mammillae (*arrow*) and reduced palisade layer.

<u>Inner surface with membranes removed</u> (168X)

C & D. Recent eggs have somewhat smaller pores (*arrows*) and larger mammillae.

<u>Tangential view with cuticle removed</u> (840X)

E & F. Recent eggs have a crystalline rather than spongy texture.

pelicans, and cormorants. The frequent occurrence of unhatched eggs in nests containing well-developed young falcons and the frequent collapse of brown pelican eggs indicate that the relative importance of egg breakage *per se* varies from species to species, and that eggs which fail may have sound shells. Embryonic death in eggs which fail usually occurs early in incubation. However, chlorinated hydrocarbons are absorbed late in embryonic development (Cooke, 1971). The injection of chlorinated hydrocarbons into normal chicken eggs prior to incubation, at levels many times those encountered in most affected bird species, does not reduce hatchability (Dunachie and Fletcher, 1969; Guthrie and Donaldson, 1970). Hence, it would appear that some characteristic of the shell other than thickness, or some factor other than the DDE content of the yolk lipids is responsible for death of the embryo.

This is the first study of the relationship of eggshell quality, as measured by BBS, to reproductive success in a wild population. It has shown that a significant correlation does exist between shell quality and nesting success in the common tern. Enderson and Berger (1970) have shown that a similar relationship existed between thickness index and nesting success in a manipulated prairie falcon population. Eggshell quality reflects both chemical and structural properties of the shell. Hence, this does not imply that a relationship necessarily exists between nesting success and thickness *per se*. Apparently it is a structural or chemical property, or combination thereof, which is important.

The eggshell serves as a rigid container, a calcium source, a microbial barrier, and the principal resistance to gaseous exchange for the embryo. Denting and hence altered rigidity accounted for only 10 percent of the tern eggs which failed. There was no difference in the calcium content between shells of viable tern eggs and those of eggs which failed. No consistent pattern of bacterial contamination existed in eggs which failed and only 50 per cent of the eggs which failed showed any sign of microbial contamination. Thus, the rigidity, calcium content, and antimicrobial properties of the majority of tern eggs which failed did not differ appreciably from viable eggs, leaving their respiratory properties suspect.

The respiratory properties of the shells of eggs which failed were markedly different from those surrounding viable embryos, and were not associated with any significant thinning. There is evidence that reductions in oxygen tension can induce death of embryos of domestic fowl in the first 48 hours. It does not seem unreasonable to assume that such a reduction could in fact account for most of the embryonic mortality observed in the Buffalo Lake colony. Peakall *et al.* (in press) induced changes in evaporative water loss of eggs of three species representing three orders by incorporating DDE in the diet. This then is a mechanism by which DDE can affect eggshells in such a way as to induce embryonic mortality which is independent of shell thinning. Several investigators have noted that at a specified residue level, hatchability is independent of thickness *per se*. In the common tern this mechanism is operative at residue levels which are lower than those associated with marked shell thinning. It would appear, in this species at least, that reduction in pore area available for respiration precedes marked thinning and is probably far more significant in reducing nesting success.

There are indications that the reduction in pore area is the result of abnormalities in the biomineralization process which reflect abnormalities in the organic matrix of the shell. The changes in the organic matrix reflect changes in protein metabolism. To the author's knowledge, there have been no studies of the effects of chlorinated hydrocarbons on the protein metabolism of birds. Studies of fish (Mehrle *et al.*, 1971) and mammals (Wassermann *et al.*, 1973) indicate that organochlorines have a marked effect on serum amino acid, albumen, and globulin concentrations. There is evidence that stresses such as increases in environmental temperature can induce changes in matrix composition in shells of domestic fowl (Cooke, 1973). Hence, the protein metabolism of birds is a labile system. It is entirely independent of carbonic anhydrase, the inhibition of which is responsible for decreased calcium carbonate deposition and shell "thinning."

Figure 13 is an attempt to indicate the mechanisms and interrelationships between factors involved in the "thin-eggshell syndrome" as we know them at the present time and as they are seen in the common tern. The relative importance of each mechanism will depend on the DDE concentration and interspecific variability in sensitivity to this pollutant. The roles of heavy metals, PCB's, and other organochlorines are not clear. There is good laboratory evidence that all indicated mechanisms leading to reduced hatching success are in existence. Whether they are functional in all species remains to be determined.

It is intuitively obvious that continued recruitment at a rate below that necessary to maintain stability will result in a population decline. Such declines are ~~well~~ documented in several species of raptorial and piscivorous birds. The rate at which affected populations recover when the level of environmental pollution decreases should depend

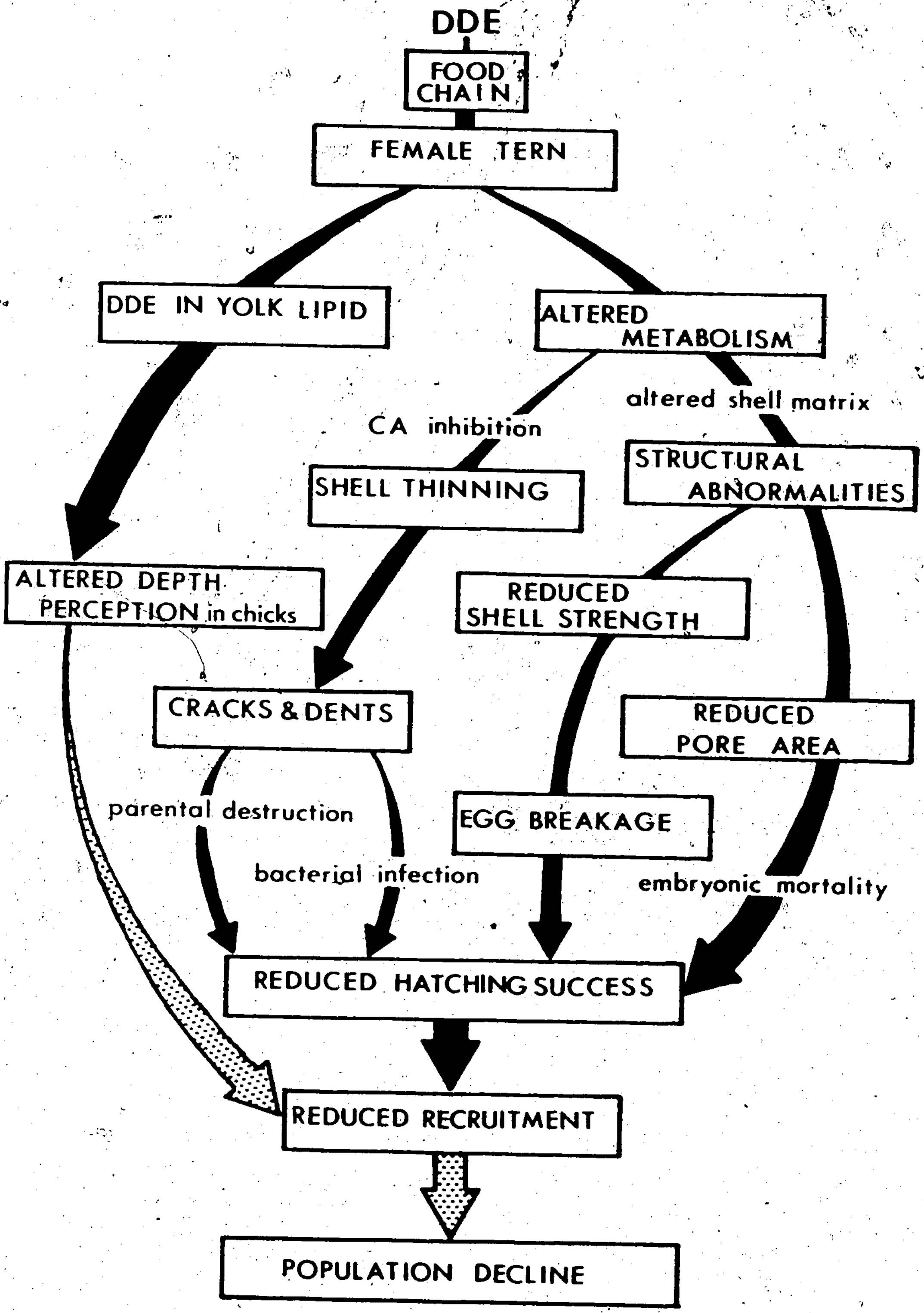


Figure 13. The mechanisms and interrelationships of factors by which organochlorines are thought to affect reproductive success in the common tern. Solid arrows indicate mechanisms which have been observed in the Buffalo Lake colony and shaded arrows represent postulated outcomes.

upon which mechanism is most important in the decline of the species in question, and the species-specific threshold for that pollutant. If this is so, we can expect DDE-sensitive species such as the common tern to continue to show reduced reproductive success for a longer period than less sensitive species such as the peregrine falcon. The colonial habits, sensitivity, wide distribution, and ease of study make the common tern an ideal indicator species for monitoring organochlorine pollution.

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APPENDIX 1

MODIFIED METHOD OF HARDWOOD *ET AL.* (1969) FOR THE DETERMINATION OF PHOSPHORUS IN EGGSHELL

Reagents:

4.0 N sulphuric acid

10% w/v ascorbic acid

0.96% w/v ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$

0.267% w/v potassium antimony tartrate $KSbOC_4H_4O_6$

Standard (5 mg P/100 ml): Dissolve 22 mg of KH_2PO_4 in 70 ml of distilled water. Add 5 ml of 1.0 N sulphuric acid and dilute to 100 ml.

Method:

1. Add 1.0 ml of digest to a 10 ml volumetric flask.
2. Add 1.0 ml of 4.0 N sulphuric acid. Mix.
3. Add 1.0 ml of molybdate reagent. Mix.
4. Add 0.8 ml of 1:1 ascorbate:tartrate reagent prepared immediately before use. Mix well.
5. Dilute to 10.0 ml with deionized distilled water. Mix well.
6. Allow color to develop for 10 minutes at room temperature.
7. Quantify in a spectrophotometer at 890 nanometers against a reagent blank and standards.

Standards are run in parallel with all samples. Standards are treated as above but 0.2 ml (10 μg) and 0.5 ml (25 μg) are substituted for the 1.0 ml of digest.

APPENDIX 2

CLEAN-UP PROCEDURE FOR EGG CONTENTS AND FOOD ITEMS

Loosely capped quart sealers containing 60/100 mesh Florisil previously activated at 1200 C were reactivated at 300 C for a minimum of 12 hours. The Florisil was partially deactivated with 3 percent redistilled water by weight and kept in an air-tight, rubber-sealed quart sealer until used.

Fifty grams of Florisil was placed in a 28 x 600 mm Pyrex column and pre-washed with 100 ml of a 50:50 methylene chloride:redistilled petroleum ether (BP. 38.0 to 49.6 C) solution. The equivalent of 5 g of homogenized fresh egg contents, or 5 g of homogenized food items was added to an additional 50 g of Florisil and ground in a mortar until a free-flowing powder resulted. This powder was then added to the top of the column above the previous 50 g of Florisil.

Two consecutive 100 ml portions of a 1:4 mixture of methylene chloride:redistilled petroleum ether were used to rinse the glassware and utensils and were added along with a further 400 ml of this mixture to a 1 liter reservoir fitted in the top of the column and allowed to elute.

The eluate was flash-evaporated to dryness. Ten milliliters of spectroanalyzed n-hexane was added to the flask and the resulting solution placed in a pre-cleaned, labelled test tube with foil-lined screw cap. The tubes were stored at -15° C until gas chromatographic analyses were carried out.