THE ANNUAL REPRODUCTIVE CYCLE OF THE CALIFORNIA GULL
II. HISTOLOGY AND FEMALE REPRODUCTIVE SYSTEM

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The first part of this paper dealing with the criteria for age determination in the California Gull (*Larus californicus*), with the size of the testes and seminiferous tubules, and with the stages of spermatogenesis appeared in the preceding issue of the Condor (Johnston, 1956). The conclusion of this work concerns further phases of the male reproductive cycle, namely, intertubular histology of the testis, incubation patches, and subadult breeding, and it presents data on the female reproductive cycle. Also included in Part II is a general summary.

INTERTUBULAR HISTOLOGY OF TESTIS

Several different kinds of intertubular cells have been described from several different species of birds, and there is limited agreement among various authors as to the morphology and physiology of the intertubular cell types. Some of these differences in opinion undoubtedly stem from the fact that different species have been studied. Other authors, however, working in a less detailed fashion, have merely referred to the presence or absence of secretory interstitial cells without reference to the other morphological types present, but the recent work of Marshall (1949) indicates that the complete morphological picture of intertubular tissue must be understood before the secretory nature of the cell types can be correctly interpreted.

At least two detailed studies of intertubular histology have been carried out recently: on the White-crowned Sparrow by Blanchard and Erickson (1949) and on the Fulmar by Marshall (1949). These authors recognized six or more different morphological cell types in the intertubular areas in each of these species, and it is possible to equate some of these types between the two species. Microscopic examination of testes from the California Gull revealed the fact that most if not all of the intertubular cells in this species were rather similar to those described and illustrated by Marshall for the Fulmar. Since Marshall utilized special stains to differentiate some of the cell types, it was not possible in all cases to compare directly the different kinds of cells between the Fulmar and the California Gull. Furthermore, in the gull material no attempt was made to relegate connective tissue cells to the detailed subgroups which Blanchard and Erickson recognize.

In the California Gull the following kinds of intertubular cells were identified:

1. *Melanoblast.* These cells may vary in size and shape but generally are large and dendritic. At times, especially during the winter and in subadult birds, the melanin granules tend to obscure other intertubular cells as well as the structure of the melanoblast itself.

2. *Juvenal interstitial cell.* This kind of cell was not investigated as thoroughly with special stains as it was in Marshall's study. It is generally recognized by its relatively small size and rounded appearances of cytoplasm and nucleus. As Marshall points out, this type is common in immature birds and adults not in breeding condition. Presumably, these cells develop into the next type. If they do, there is a great mortality because the mature cells are never as numerous as the juvenal ones.

3. *Mature interstitial cell.* It was sometimes difficult to decide whether a given cell was juvenal or mature due to gradations in size, but a typical mature cell is larger than any other intertubular cell type. Usually, in breeding adults the mature cells are clumped together in groups up to about ten, but occasionally a single cell is wedged in the intertubular spaces. Some cells identified as this type, on the basis of size alone, might have been the fuchsinophil type found by Marshall and others.

4. *Connective tissue cell.* This is the most consistent type of cell found in the intertubular spaces and is at times the dominant cell type present. These small cells have spindle-shaped nuclei and cytoplasm and are about the same size or perhaps a little smaller than the juvenal interstitial cells.

Many of the standard works which deal with avian reproductive cycles and the histology of testes have emphasized the cells which secrete androgens. Marshall's interpretations seem to be the most plausible at the present time, primarily because he has combined special staining techniques with analyses of the morphological cell types and has correlated these findings with the breeding biology of the bird. He believes the juvenal interstitial cells are found primarily in the nonbreeding season and that these cells develop into mature lipoidal secretory interstitial cells during the breeding season. His interpretation of the secretory cells is diametrically opposed to the views of Sluiter and van Oordt (1947) who believe that the lipoid cells do not secrete androgens but that a nonlipoid cell, "secretory cell B," is the source of androgens. Contemporary knowledge of the chemical structure of androgenic steroids favors Marshall's view.

Seasonal changes in the size of interstitial cells occurred in the form of a gradient, beginning in late January and culminating in the largest cells at the height of the breeding season. These observations would seem to support Marshall's contention that the juvenal interstitial cells increase in size, becoming largest and secretory during the breeding season.

Mature interstitial cells have been counted or estimated in a variety of different ways in many vertebrates, but, since only relative abundance was desired, a simple method has been utilized in this study. The criteria of relative abundance used by Blanchard and Erickson (1949:268) for all intertubular cell types have been adopted for the gull material with only a few changes. These criteria are as follows:

1. Abundant—comprising all of the intertubular area except for a few other cells.
2. Common—the predominant intertubular cell type. About ten cells visible in any field of a given section at 400×.
3. Fairly common—one to three cells visible in any field of a given section at 400×.
4. Occasional—five to ten cells in a given section.
5. Rare—one or two cells in a given section.
6. Absent.

None of the cell types was ever recorded as abundant, but in certain instances connective tissue cells or juvenal interstitial cells approached this category. The most difficult distinction arises in trying to decide when the cells are rare or absent. Considerable searching might reveal only one cell in a section, while diligent search of material from another bird with essentially identical testes might reveal no cells. The difference here might be due only to sampling technique. Physiologically, there is probably little difference between these two stages.

A graphic presentation of the mature interstitial cell cycle is presented in figure 1. For all practical purposes, the mature interstitial cells are absent during the winter months.

Intertubular areas in winter in all age groups are characterized by having a predominance of connective tissue cells and juvenal interstitial cells. Only an occasional capillary can be found. Variable amounts of melanin are present and, depending upon size of the testis and age of the bird, impart a black or brown color to the entire testis. Frequently, these melanin deposits obscure the quantity and quality of intertubular cells, especially in first-year birds when the melanin is concentrated. These findings are in general agreement with the observations of Marshall and other workers who have reported correlations among melanin deposition, size of the testis, and gross color of the testis.

Mature interstitial cells first begin to appear in January in all but first-year birds. Concurrently, other changes in the intertubular areas occur as the breeding season approaches. The once abundant melanin deposits gradually disperse or disappear, at least

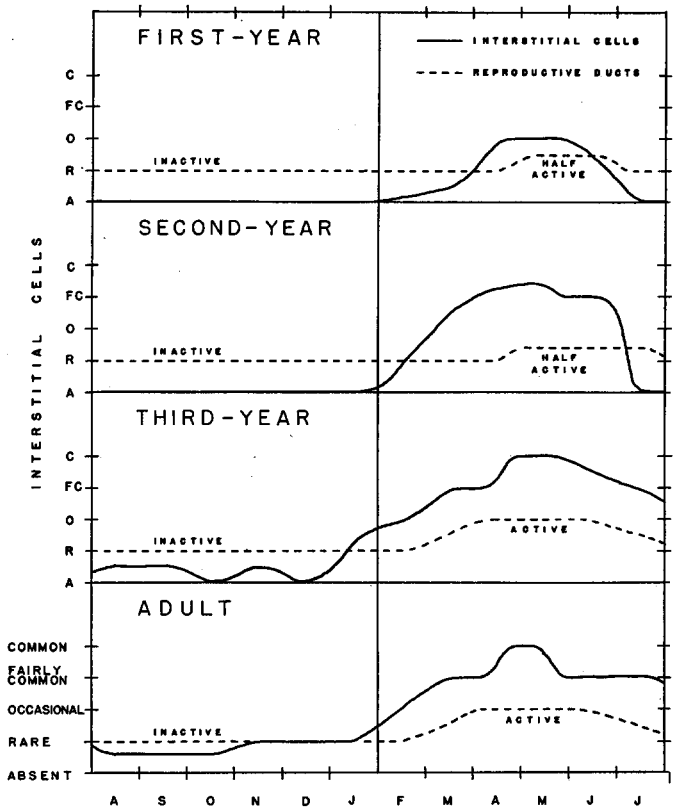

Fig. 1. Correlation between relative abundance of mature interstitial cells and activity of reproductive ducts.

in birds with the largest testes, until at the height of the breeding season no more melanin can be found either in intertubular areas or in the tunica albuginea. As the mature interstitial cells increase in number, the juvenal ones decrease and are entirely absent when the mature type reaches its greatest peak of abundance. No visible differences, except in relative abundance, could be detected among the interstitial cells of the various age groups of the gull. Breeding and regressing testes have many intertubular capillaries.

Several further facts are revealed in figure 1. First-year and second-year birds never have as many interstitial cells as the other age groups, this fact being correlated with less developed reproductive tracts and duller coloration of the soft parts. Second-year birds, however, during the spring months have more interstitial cells than first-year birds. Third-year and adult birds are essentially alike in numbers of these cells present, and there are no differences between the nonbreeding and breeding third-year birds.

In many aspects the interstitial cell cycle of the California Gull follows closely cycles described for other birds. First-year Fulmars, according to Marshall, have mostly juvenal interstitial cells, whereas adults have increasing numbers of mature interstitial cells as the breeding season progresses. Similarly, in the British Starling, Bullough (1942:174–180) has described an interstitial cell cycle which begins with first-year birds having "not common" interstitial cells when their testes are at maximum size

(80.1 mm.^{3}; both testes combined). During the winter months, these cells are rare or absent, but, when the adults come into breeding condition (testis mean 3988.2 mm.^{3}; both testes combined), the interstitial cells are common. As in the gulls, a correlation may be established among number of interstitial cells, size of reproductive ducts, and intensification of bill coloration.

In male Red-winged Blackbirds, Wright and Wright (1944:53) state that "Leydig cells appear to be absent when the testes are at a maximum, during regression and during the inactive period." But this is not generally true of birds. The various studies already mentioned for the White-crowned Sparrow, Oregon Junco, Fulmar, and Starling have shown that interstitial cells are present in various quantities at the height of the breeding season. Similarly, in the California Gull, it has been shown that mature interstitial cells are present in third-year and adult birds from at least February until July. As Wright and Wright indicate, it is generally true that in birds the intertubular material is relatively less abundant than tubular material in testes of maximum size. At this time, however, at least in most species which have been studied thoroughly, interstitial cells may occupy much of the intertubular area.

In the California Gull, as in other species of birds which have been studied, there is a direct correlation between the maximum number of adult interstitial cells and maximum development of the reproductive ducts. Bouin and Ancel (1903, 1904) first " . . . demonstrated that only when the interstitial cells were large and filled with lipid droplets were the secondary sex organs well developed; that in species with seasonal sexual activity, the secondary sex organs developed concomitantly with the interstitial cells, whereas the activity of these glands bore no constant relation to the condition of the spermatic tubules. . . . " (from Deane and Seligman, 1953:176). Although this generality exists for vertebrates, it has nevertheless been shown that an inverse relationship occurs in the garter snake (Fox, 1952:532) and the lump-nosed bat (Pearson, Koford, and Pearson, 1952:286).

Oslund (1928:263) maintains that there is an increase in the number and volume of the interstitial cells in birds during the period of sexual *inactivity,* but this point of view is not documented by factual evidence. As many other authors have shown, intertubular material is at a maximum abundance during sexual inactivity, but at this time secretory interstitial cells are absent, these appearing only with the onset of sexual activity.

REPRODUCTIVE DUCTS

In the California Gull it was not possible to preserve and section all parts of the reproductive ducts (rete testis, vas efferens, epididymis, ductus deferens and seminal vesicle), but many of the sections of testes also contained portions of the rete testis and epididymis. At least one sample of these structures was examined for each age group for each month, and, whenever two or more birds of the same age were available, they always had ducts in comparable stages of activity. Microscopic examination revealed histological details essentially similar to those studied and figured by Bailey (1953) in the Oregon Junco.

Several investigators, notably Bailey (*op. cit.*) and Riddle (1927) have shown that these ducts are under androgenic control. They hypertrophy in the breeding season, but shortly thereafter involute to a small and inactive condition in fall and winter. As Bailey demonstrated by laboratory injections in caged juncos, they enlarged under the influence of androgenic substances but not under estrogenic or progestational substances in a manner similar to that found in comparable wild birds of the same species.

Fox (1952) and others have used as a criterion of enlargement actual measurement

of the height of the epithelium of the ducts while Bailey used, in part, weight of the duct section. In the microscopic examination of gull material, the use of relative sizes was found to be sufficient to determine whether or not these structures were active. From Bailey's work the following criteria were adopted.

	Inactive	Active
Rete testis	flattened tubes; epithelium a single layer of closely packed columnar cells.	enlarged tubes; epithelium of flattened to low cuboidal or squamous cells.
Epididymis	circular tubes; practically no lumen; epithelium a single layer of 7–10 wedge-shaped cells.	enlarged tubes; greatly expanded lumen; epithelium pseudostratified ciliated columnar.

There was almost always a direct parallel between the size and activity of the rete testis and that of the epididymis; when one was active and large, so was the other. This would suggest that the other portions of the reproductive ducts which were not examined were in similar condition. As a partial exception to this statement, occasionally a bird with regressing testes in June or July would have one of these structures active while the other was inactive. This condition, however, is plausible since it is perfectly possible and probable that these structures do not enlarge and involute necessarily at the same rate, especially since they are of different sizes. Thus, in figure 1 "activity" and "inactivity" refer to either or, more frequently, to both rete testis and epididymis. Even in the absence of precise measurements, it was possible to recognize readily an intermediate condition which has been termed "half active." This condition of approximately one-half enlargement was especially apparent in first- and second-year birds in May.

The graph of duct activity (fig. 1) shows the same general trend described by Bailey and Bullough in their respective studies, at least as far as third-year and adult birds are concerned. If one assumes the same endocrine-target organ relationship in this gull that Bailey demonstrated in the juncos, then it can be stated that during the breeding season in these two age groups there is an increase in androgen output with a resulting increase in size and activity of the reproductive ducts. Furthermore, there is good evidence from the number of adult interstitial cells present and from soft-part colors that in first- and second-year birds there is enough androgen production to bring these ducts into a partly active state.

As I have indicated, when testes are regressing in size, there is some variation in activity of the reproductive ducts from one bird to another of the same age group, this being especially true of postbreeding adults. On June 19 and 20, 1953, for example, of seven adult males examined (left testis ranging from 104 to 321 $mm.^3$), two had ducts in the active state, one was half active, and four were inactive. By the end of July, however, when regression is essentially complete, all adults have inactive, small ducts. There is a general, though by no means absolute, correlation between size of the regressing testis and size of the ducts: testes below 200 $mm.^3$ usually are associated with inactive ducts.

HISTOCHEMICAL TESTS

Recently, histochemical methods have been utilized to localize the sites of production of the male sex hormone. A review and critique of these methods can be found in the paper by Deane and Seligman (1953). Even though the results at present may not be entirely conclusive in all instances, the gull material was subjected to two techniques in an attempt to demonstrate by color reactions that the interstitial cells are producing androgens at least at some time during the annual cycle.

The 2,4-dinitrophenylhydrazine reaction.—This test has been modified from its original form and described in detail by Albert and Leblond (1946): It is based upon the formation of yellow hydrazones by treating the tissue with 2,4-dinitrophenylhydrazine. Various investigators (for example, Pollock, 1942) prior to Albert and Leblond had used this same general reaction to demonstrate the presence of ketosteroids in parts of the mammalian adrenal cortex, interstitial cells of the testis, parts of the ovary, and the human placenta.

Tissues should be fixed in 10 per cent neutral formalin, but all the gull testes were routinely fixed in nonneutralized (acidic) formalin. Thus, it was necessary to determine at the outset whether or not the difference in pH might give different results. The right testis from a laboratory mouse was fixed in neutralized formalin while the left testis was placed in nonneutralized formalin. Frozen sections of both testes were cut, and these were treated with 2,4-dinitrophenylhydrazine. Both gave the same strong positive reaction (intense yellow color imparted to granules and/or droplets in the interstitial cells; no localized color in tubule constituents). Routinely using the mouse testis fixed in nonneutralized formalin as a control, the test was then conducted on selected gull testes.

The mouse testes had been in the fixative for at least one month, but the period of fixation for the gull testes was from seven to twelve months. This variation in the method may not be important, inasmuch as Ashbel and Seligman (1949:567) indicated with a related technique that apparently a duration of fixation from several hours to several months did not affect the results.

All the gull testes selected for this test were from the right side of adult birds, one taken each month from February through May. This sample was intended to include testes in all spermatogenic stages, from minimum to maximum volumes, and with various numbers and activity of adult interstitial cells. Within this one age group, this sample should have included birds with minimum to maximum androgenic titers. The results of this test on gull testes were negative throughout whereas positive reaction was seen in the mouse material.

Few published data are available for this test on avian testes. Levine (MS) applied the technique to about 30 species of birds from California; this sample included breeding and postbreeding adults and immature birds. The results on testes were mostly negative with the highly dubious exception of two birds which were classed as weakly positive. Arrington, Fox, and Bern (1952), working with chick and sexually mature rooster testes, reported a slightly positive reaction for the chick and a strongly positive reaction for the rooster. In both of these studies, however, I would disagree that positive reactions were obtained. To date, we must conclude that no one yet has satisfactorily demonstrated the presence of ketosteroids in the avian testis with this technique.

The hydrazide-tetrazonium reaction.—Since the development of the dinitrophenylhydrazine test, considerable question has been raised by various workers as to its specificity. In order to improve upon the sensitivity of the colored hydrazine reaction, Ashbel and Seligman (1949) synthesized new compounds and devised a new method which depends upon the coupling of a hydrazide with a tetrazonium compound. The dinitrophenylhydrazine reaction had already been used to demonstrate the presence of carbonyl groups of ketosteroids in various endrocrine glands, and Ashbel and Seligman, using the new technique, were able to duplicate these results. The testes of a rat, rabbit, and dogs were stained for the lipoidal carbonyl groups with the result (positive reaction) that fat droplets in the cytoplasm of the interstitial cells stained blue. The nucleus was unstained and was outlined by the surrounding blue droplets.

Before the gull testes were subjected to this test, laboratory mouse testis was again

run as a control. Positive results resembled those figured in color by Ashbel and Seligman (1949: fig. 2). Comparable positive results on the mouse testis were obtained by using either their original method or by a shorter modification described by Deane and Andrews (1953). Selected gull right testes were then run concurrently with the mouse material (control) using the short method. A larger series (19) of gull testes was used than in the previous test: a representative was taken from each age group in order to cover, as far as possible, the entire range of important spring cyclic events, that is, from the winter inactive condition through gonadal recrudescence to the beginning of regression of the testes. The results of this test for the gull testes were all negative, even though the control mouse material was always positive.

The same test has been applied to a number of vertebrates by Ashbel, Cohen and Seligman (1951). Various vertebrates (frog, rat, mouse, and other mammals) showed a positive reaction in the interstitial cells, but only a few, such as the pig, showed positive Sertoli cells. Their results were negative for two adult roosters taken in August. The only positive reaction obtained was a sudanophilic test on the interstitial cells. Since roosters are not necessarily cyclic, it is difficult to understand why they were negative, for the ketosteroid test with all the other vertebrates was positive. Other investigators, such as Marshall (1949), have also demonstrated that adult functional avian interstitial cells are positively sudanophilic.

As in the dinitrophenylhydrazine reaction, it is necessary to conclude here that positive histochemical evidence for the presence of ketosteroid in the avian testis is lacking.

INCUBATION PATCHES AND SUBADULT BREEDING OF MALES

None of the first- or second-year birds which were collected at Mono Lake ever possessed incubation patches, and, since this structure was an adequate criterion of breeding, it is safe to state that birds of these two age groups do not breed at this colony. Furthermore, there is no evidence in the literature that they breed at any other colony.

The situation in third-year males is complicated by the fact that some birds breed while others do not. From May 15 to 17, 1953, all adults had three well-developed incubation patches. During the same period, three third-year males had incubation patches while three did not. One of the males which had these structures was shot as it incubated two eggs (two- and three-day-old embryos); all the other birds were collected as they flew over the colony.

From May 29 to 30, 1953, again all adults had incubation patches. Of six third-year males, four had these structures. Three of them had incubation patches that were noticeably smaller than those of adults collected on the same day. On June 19, 1953, all adults had refeathering incubation patches, and similar structures were found in one out of two third-year males. These records indicate that more than one-half of the third-year males collected in May and June at Mono Lake possessed incubation patches and were undoubtedly breeding.

Considering the fact that some third-year males develop incubation patches while others do not and the fact that their patches are frequently smaller than adults', it is probable that there are different hormone titers in different third-year males. This belief stems primarily from the investigation of Bailey (1952) on the incubation patch of birds. In adult California Gulls he found that nonbreeding, premigratory birds of both sexes in April had virtually no prolactin in their pituitaries, but at the height of the breeding season in May they showed a much greater prolactin content. Further experimentation proved that both prolactin and estrogen are necessary for the formation of the incubation patch and that nonbreeding birds will develop this structure if these two hormones are injected. Thus, in the third-year gulls of the present study we can postu-

late that one or both of these hormones are at subthreshold level in those birds which do not breed and that breeding third-year birds elaborate threshold quantities but still in varying amounts so that different sizes of incubation patches result.

The evidence from other gulls shows that only a limited number of subadults breed in some species. This has been demonstrated for the Herring Gull, *Larus argentatus* (Gross, 1940; Lincoln, 1928) and the Black-headed Gull, *Larus ridibundus* (Steinbacher, 1936; Kirkman, 1937). It is likewise true of these and other species, for example, the Little Gull (*Larus minutus*), that subadults may return to the breeding colony and yet not breed (Brouwer and Haverschmidt, 1942). In fact, Tinbergen (1936) observed that immature Herring Gulls may pair, build a nest and copulate, but still fail to lay eggs.

The phenomenon of nonbreeding subadults is described for several species of sea birds by Wynne-Edwards (1939). In certain species, such as the Gannet (*Moris bassana*), Kittiwake (*Rissa tridactyla*) and Fulmar (*Fulmarus glacialis*), some or all of the subadults may return to the breeding colonies and yet not breed. Although he believes that most subadult Pomarine Jaegers (*Stercorarius pomarinus*) do not return to the breeding colonies but remain on the wintering ground several thousand miles to the south, the recent work of Pitelka, Tomich, and Treichel (1955), has shown that many subadults of both sexes may be found breeding at Barrow, Alaska. The breeding pair might be composed of two subadults, one subadult and one adult, or two adults.

THE OVARY

In previous studies of avian reproductive cycles, few data have been present for female birds of wild species. Some investigators, however, have shown that females undergo cyclic changes in the ovary and associated structures which are as striking and significant as the better-known cyclic changes in the male system. Notable among these have been Bullough (1942) and Stieve (1919), both of whom emphasized microscopic aspects of the ovary, and Paludan (1951) who concentrated on macroscopic observations as correlated with breeding biology. In the present work on the California Gull (*Larus californicus*) the data amassed for females, although somewhat less complete than those for males, revealed cyclic phenomena that were roughly comparable to the male cyclic events. The general features of the breeding cycle at the Mono Lake colony in California in 1952 and 1953 have already been described and diagrammed (Johnston, 1956: fig. 1).

In the California Gull the ovary varies in size from season to season primarily due to the growth and enlargement of the follicles. During the nonbreeding months for all age groups, the ovary was 12 to 17 mm. long and 6 to 11 mm. wide. There was some tendency for birds in the first-year plumage to have smaller ovaries than those of older birds, but the difference was not significant or constant. The ovary varied from a white or pinkish L-shaped organ to a flat, elongate body. Due to these irregularities in size and shape, volumetric determinations based on measurements comparable to those carried out on testes were impossible, and weights of the organs were not taken. Seasonal variation and development of the ovary, therefore, were based on the size of the largest follicle as determined by caliper measurements, along with partly subjective observations on size and activity of the associated oviduct and, when available, information on incubation and incubation patches. A similar method was employed by Bullough (1942: 199 ff.) except that his study was almost entirely microscopic in nature; most of his measurements were made of oocyte diameters taken with an ocular micrometer from prepared sections of the ovary.

Unless otherwise specified in the discussion to follow, all birds taken during the

breeding season were collected at random as they flew over or near the breeding colony, and, where general references are made to breeding, these are based on average conditions for the entire breeding colony and not for a specific bird.

A summary of seasonal follicular development for all age groups is presented in figure 2. Throughout the entire first year, all follicles were less than 1 mm. The absence of any enlarged follicles, especially during the breeding season, was noticeable. Toward

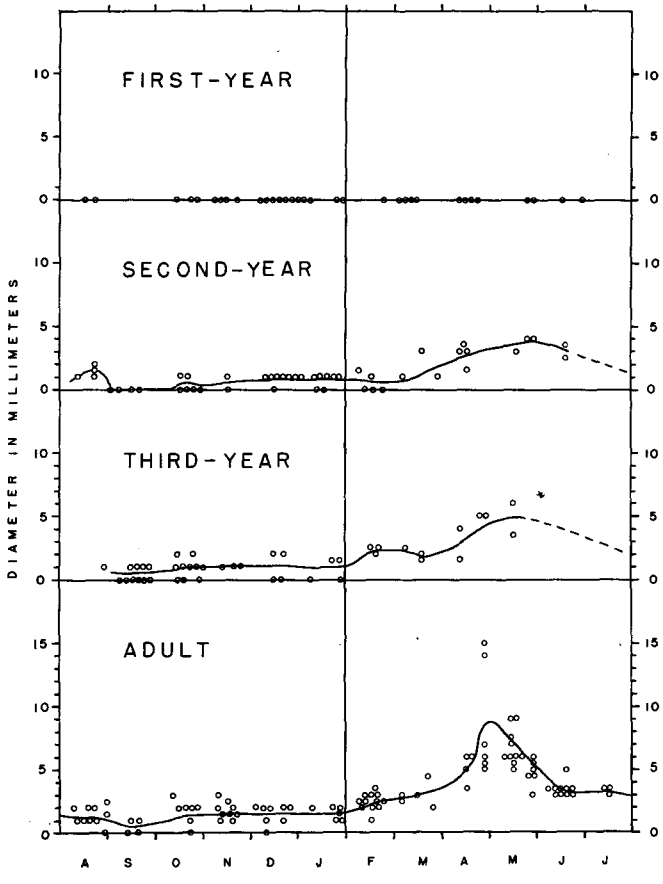

Fig. 2. Cycle of maximum follicle size. Solid line represents mean value; broken line, suggested trend.

the end of the second summer and early fall, however, there was a tendency for follicles to become larger, so that during the second winter a mean follicle size of 1 mm. was achieved which differed noticeably from the condition found in first-year birds in the same month. Beginning about the end of March in second-year birds, there was a gradual increase in size of follicles until a mean maximum of 3.5 mm. was reached about June 1. Since no second-year females were obtained in late April and early May at the breeding colony, it is possible that some birds might have had larger follicles in this period, but my data for late May are not significantly different from comparable data on birds taken in early and mid-May at Great Salt Lake, Utah. After June 1, these somewhat enlarged follicles apparently become atretic so that, by the end of August,

birds now in the third-year plumage had follicles of only about 1 mm. There was no evidence that the relatively few first- and second-year females that were collected at the breeding colony ever bred.

During the third winter the mean maximum follicle size was about 1 mm. and thus was similar to conditions found in second-year birds, although there was some tendency for follicles to be larger. Only an occasional winter second-year bird had a follicle which was over 1 mm., but several third-year birds had follicles up to 2 mm. Whereas spring enlargement of follicles in second-year birds began in late March, in third-year birds there was a considerable upswing of follicle size in mid-February. This growth of follicles continued until a mean maximum of 5 mm. was reached at the end of April. No third-year females were obtained in June or July.

Adult birds (this group included birds which had just molted from third-year to adult plumage) that had recently returned to the San Francisco Bay area in August had ovaries in an inactive condition, the mean maximum follicle size being about 1.5 mm. There was a general tendency for follicles in winter adults to average 1.5 to 2.0 mm.; thus they were somewhat larger than in birds of other age groups at this time of year. As in third-year birds, there was a noticeable average increase in activity of the ovary and follicular development in mid-February, with the curve of follicle size rising abruptly to a mean of about 8 mm. at the end of April on the breeding grounds.

On April 28, 1953, two of several adults collected had follicles which measured 14 and 15 mm., respectively. One of these birds had three follicles of 7 mm., and the other had several 6 mm. follicles. At this time no nests had been constructed at the colony and most birds had no trace of an incubation patch. At the time in the field, I thought that these largest follicles were about to be ovulated, but subsequent consideration has shown that such a view may be wrong. In the first place, in the Herring Gull, admittedly a somewhat larger bird, Paludan (1951:73) found that an adult fully two days before laying its first egg had follicles 36, 32, 27 and 19 mm. In the second place, the work of Bailey (1952:125) and others has indicated that defeathering for the incubation patch generally occurs several days before the first egg is laid. In only one of these two gulls was there even a trace of defeathering. Probably ovulation was still several days off. The mean maximum follicle size, therefore, given for adults in the breeding season should probably be much greater.

In 24 adult females collected in the breeding period at the end of April and during May, it was possible only rarely to observe recently ovulated or collapsed follicles, but my failure to observe more of these was due in part to my absence from the colony when most of the eggs were being laid in early May. It was impossible to check the nest contents of most of the birds collected, but the following females were shot from nests the contents of which were then examined and were correlated with reproductive condition:

May 15, 1953	3 collapsed follicles, others up to 6 mm.	incubating 2 eggs, 2 days old
May 15, 1953	1 collapsed follicle, others up to 7.5 mm.	incubating 2 eggs, 2 days old
May 15, 1953	2 collapsed follicles, others up to 7 mm.	incubating 2 fresh eggs
May 29, 1953	no collapsed follicles, others up to 4.5 mm.	incubating 2 eggs, 3 weeks old
May 29, 1953	no collapsed follicles, others up to 3 mm.	incubating 3 eggs, 3 weeks old
May 29, 1953	no collapsed follicles, others up to 5.5 mm.	incubating 3 eggs, 2 weeks old

From these data it would appear that ovulated follicles were resorbed rather rapidly, probably within a week, even though some enlarged follicles were still present. This subject has been treated in detail by Paludan (1951:49) who noted that the Herring Gull had no collapsed follicles visible on the surface of the ovary nine days after the last egg was laid, and by Stieve (*fide* Paludan, *op. cit.*: 57) who reported that some follicular degeneration began after the second egg was laid in the Jackdaw (*Corvus monedula*). On the other hand, some follicles showed no signs of degeneration even after four weeks of incubation by the female. In the British Starling, Bullough (1942:207–209) found microscopically what he has called corpora lutea in both first-year and adult birds; these persisted for about two months after incubation.

SIZE AND ACTIVITY OF THE OVIDUCT

There is a direct correlation between size and activity of the oviduct and the development of the follicles. During the winter months in all age groups the oviduct remained small and inactive. Toward its proximal end it always measured less than 1 mm. in width. In the first breeding season, there was only a slight enlargement to 1.5 or 2.0 mm., but there were no signs of reproductive activity. This condition of the oviduct was the same for birds in their second breeding season even though some of these birds had somewhat enlarged follicles at this time.

In the third breeding season, at the end of April, there was an enlargement of the oviduct to 3 mm. in width, this being true of birds the ovaries of which contained follicles up to 5 mm. in diameter. This was the most advanced condition discovered in any third-year females at any time in the breeding season.

As in the case of mean maximum follicle size, in breeding adults there was a rather abrupt enlargement of the oviduct following the inactive winter condition, and at the same time it became more convoluted and twisted. On April 27 and 28, 1953, oviducts varied from 2 to 6 mm. in width, a variation which was correlated with maximum follicle size and development. On these days birds with the smallest follicles (maximum 5 to 7 mm.) had the least developed oviducts (2 to 4 mm.). The two adults which had the largest follicles had oviducts 6 and 7 mm. wide, the greatest width found in any birds collected.

In the middle of May, after adults were incubating eggs, there was a noticeable collapse and subsequent regression of the oviduct, although an occasional bird continued to show near maximal development. By the end of May when most of the eggs were at least two-thirds incubated, most of the birds had oviducts which had diminished to 3 or 4 mm., and this size was maintained as an average condition throughout June and July. Adults recently returned to the San Francisco Bay area in August had oviducts in the small, inactive condition. A similar cycle of enlargement and regression of the oviduct, in conjunction with follicular development and the breeding season, was demonstrated in the adult British Starling by Bullough (1942:209–212).

Several investigators have shown that the hypertrophy of the oviduct in birds in the breeding season is under the control of estrogenic substances. Under natural conditions, Keck (1934) showed that the maximal development of the oviduct in the English Sparrow (*Passer domesticus*) coincided with maximal development of the ovary in the breeding season, and then by injection of female sex hormone he was able to bring inactive oviducts into a breeding condition. Such results have also been demonstrated in doves by Riddle and Tange (1926).

INCUBATION PATCHES IN FEMALES

Incubation patches, when functional, were considered as evidence for breeding second only to the taking of an incubating bird from a nest. At no time was an incubation

patch found in any first-, second- or third-year females, even when some of these birds were collected at the height of the breeding season at the breeding colony. However, all adult females, collected during May and early June at the colony, with the exception of a single bird on May 16, 1952, had three well-developed incubation patches, two anterolaterally, and one central and posterior. The first indication of defeathering for the incubation patch was seen on one adult female on April 28, 1953, a bird with a 14 mm. follicle. On the same date, other adults, one with a 15 mm. follicle, had no patches.

In spite of the fact that the mean clutch size was about two, all adults, both males and females, always had three incubation patches, and there was no apparent difference in the functional condition among the various patches in the adults, even though a bird might be incubating only two eggs. It would seem that the hormonal regulative system for this structure provides a margin of safety, as it were, in case more than two eggs are incubated.

NONMIGRATORY FEMALES

Only a small sample of nonmigratory birds was obtained during the breeding months away from the breeding colonies. The data on maximum follicle size in nonmigratory females are presented in table 1. Because of the small sample, one can draw only tentative conclusions. In the first place, it must be noted that, with one possible exception, no adult females were ever collected away from the breeding colony, but undoubtedly some adult females fail to return to the breeding colonies because occasional adults may

Table 1

Maximum Follicle Size of Nonmigratory Birds

Date	First-year	Second-year	Third-year	Adult
May 6, 1953			3	
June 4, 1952	2			
July 6, 1953		2		
July 8, 1952	<1			
July 21, 1952		2		
July 30, 1952[a]			2	1.5

[a] Possibly newly arrived migrants.

be found in the San Francisco Bay region in the summer months. In the second place, if these data are compared month for month with the data presented for birds of the same age group collected at Mono Lake (fig. 2), it will be seen that there are no significant differences in maximum follicle size, although the sample size is small. None of the nonmigratory birds had an enlarged oviduct or incubation patch.

In subadult females there are no differences in follicle size between migratory and nonmigratory birds; it should be recalled that such was not the case in males. This is further indication that males of this species tend to mature sooner than do females.

BREEDING STATUS OF SUBADULT FEMALES

There has been some discussion in the literature of the breeding status of birds in the third-year plumage, and Behle and Selander (1953:253–254) present evidence that some females do breed in this plumage. Actually there is only one unequivocal record among their data, namely, a female with a 15 mm. follicle and an enlarged oviduct taken at Great Salt Lake in early May, 1952. The mere presence of a bird at a colony is not final proof of breeding, even though it might be a male with spermatozoa. Aside from

collecting a bird from an occupied nest, and not merely in the vicinity of a nest or young, the essential morphological criterion of breeding should be the presence of a functional incubation patch, a structure which is not universally present in third-year birds of either sex.

The small number of records presented by these workers (table 7, p. 251) on largest follicle size of third-year females is likewise inconclusive evidence for breeding, because the birds were past the egg-laying period, and, except for the one bird with the 15 mm. follicle, these birds do not differ in follicle size significantly from unequivocal nonbreeding second-year females collected on the same day.

As Behle and Selander found at the Great Salt Lake colony, at Mono Lake only a small percentage of third-year birds were females. At Great Salt Lake the ratio of birds in this age group collected flying over the colony was 43 males:4 females; at Mono Lake this ratio was 29:4. This fact alone would naturally decrease the possibility of obtaining a breeding female of this age group. These authors speculated that perhaps the immature females might be in the region but not at the nesting colonies. Although this might be true at other colonies, it was not true near Mono Lake. A careful search was made of lakes, streams and small-town garbage dumps near Mono Lake in the breeding seasons of 1952 and 1953, and, aside from obviously migratory birds, no individuals or groups of nonbreeders were ever located.

Although I was successful in collecting an incubating third-year male, no females of this age group were ever collected on or even near nests. This fact agrees with the data of Behle and Selander who correctly averred that the preponderance of third-year males over females, taken as flying birds over or near the colony, could not be attributed to the fact that females were incubating elsewhere.

One would expect that third-year females would have follicles about as large as those of the adults if they were breeding or were about to breed; at least this was what Bullough (1942: table 6, p. 200) found in the British Starling. Such was not the case, however, in third-year California Gulls (see fig. 2). All of these facts lend further support to the conclusion that very few of the third-year females breed.

As has been mentioned previously in connection with the cycle of the male, breeding of subadults in other species is a fairly widespread phenomenon. For many species in which sexual maturity is reached in less than one year, this is probably a nearly universal situation, but in others, in which age groups are recognizable by different plumages during the breeding season, as in gulls, it is not so universal (see Mayaud, 1941). It is worth noting, however, that Mayaud reported female Black-headed Gulls (*Larus ridibundus*) breeding in the first-year plumage. Since sexual dimorphism is absent in this species, one must assume that the birds were collected. Noll (*fide* Mayaud) believed that precocious sexual development in this species was limited to the females, but in the California Gull it is the male which is somewhat more sexually precocious.

In the British Starling, Bullough (1942:199–214) found that during the nonbreeding months the mean of maximum oocyte diameters of first-year birds was smaller than that of adults, but during the breeding months there was no significant difference between the two groups. This was due to the fact that subadult females, as in most passerines, breed in their first spring. Hiatt and Fisher (1947:538–540) reported that in the Ring-necked Pheasant there was no evidence of oogenesis in juvenal birds in the fall although there is development of the testes in males. However, both adults and first-year females in the spring showed a trend of follicular enlargement similar to that found in the adult gulls.

SUMMARY AND CONCLUSIONS

The California Gull belongs to that group of the Laridae in which four age groups may be distinguished on the basis of plumage, colors of soft parts, and length of the bursa of Fabricius (see Johnston, 1956). These groups are first-year, second-year, third-year, and adult. The sequence of molts, plumages and age groups has been recently confirmed by banding nestlings and recovering them in subsequent years. This has made it possible to relate age group to reproductive condition and migration.

During the winter birds of all ages and both sexes are in a quiescent reproductive state, but in the spring various changes ensue. In migratory birds, first-year males show virtually no enlargement of the testes in the spring, second-year birds' testes enlarge to about one-fourth of the adult maximum size, and those of third-year birds enlarge to about one-half of the adult size. The maximum testis volume is reached in adult males at about the time the eggs are laid, and the testes begin to regress immediately thereafter. Spermatogenic activity is correlated with the size of the testis since first-year birds do not develop beyond the primary spermatocyte stage whereas most of the second-year and all third-year and adult birds produce spermatozoa. Males which do not migrate have, for the most part, significantly smaller testes than those which move to the breeding grounds, but spermatogenic activity of the small sample was apparently not significantly different from that of birds of the same age group which had migrated except for the fact that no mature spermatozoa were ever detected in the nonmigrants' testes.

A somewhat parallel condition to testis enlargement is the increase in number of mature interstitial cells and increase in size of the reproductive ducts, the most advanced condition being found in adults at the height of the breeding season. The enlarged ducts are attributed to the effects of heightened androgenic titers as a result of increased activity of the enlarged, more numerous interstitial cells. Enlarged reproductive ducts are found just after the mature interstitial cells reach their peak in numbers, indicating a lag between supposed androgen production and the effect on the target organ.

In an attempt to localize possible areas of androgen production cytochemically, frozen sections of testes from gulls in various reproductive conditions were subjected to two histochemical tests, both of which proved to be negative even though mouse material run simultaneously was positive for the interstitial cells. Thus, to date no one has satisfactorily demonstrated the presence of ketosteroids in avian testes by utilizing histochemical procedures.

By the use of reproductive stage of the gonads and the presence or absence of incubation patches, it has been demonstrated that no first-year or second-year birds of either sex breed. No third-year females were found breeding at the Mono Lake colony, but occasionally they do so at other colonies. About one-half of the third-year males were found to have incubation patches, and these were believed to be breeding. Seventy-seven out of 78 adults developed incubation patches during the breeding season, thus providing evidence that virtually all adults breed .

In females, no follicles enlarged beyond 6 mm. were found except in adults just prior to egg laying, thus providing further evidence that subadult females rarely if ever breed. Subadult females which did not migrate to the breeding colony have smaller follicles than adults, but these are not significantly different from those of migratory subadults.

There is a tendency for the younger age groups to remain away from the breeding grounds, whereas virtually all adults migrate to breeding colonies in the spring. Thus, at the breeding colonies there are fewer of the successively younger age groups. These data suggest that in some fashion adults respond most highly to migratory stimuli, but, since some subadults do migrate, there must also be a differential response on the part of different individuals within a given subadult age group.

The bill, leg, eyelid, and gape show seasonal or cyclic enrichment of colors, conditions which are attributable to increased androgen production in the spring. The attainment of the adult plumage in both sexes has been shown by other authors also to be due to these hormones in other species of gulls.

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Museum of Vertebrate Zoology, Berkeley, California, August 1, 1955.