February, 1985

Reproduction in zebra finches: hormone levels and effect of dehydration

Carol M. Vleck, *University of Adelaide*
J. Priedkalns, *University of Adelaide*

The Condor 87:37–46

REPRODUCTION IN ZEBRA FINCHES: HORMONE LEVELS AND EFFECT OF DEHYDRATION

CAROL M. VLECK
AND
J. PRIEDKALNS

ABSTRACT.—Zebra Finches (*Poephila guttata*) of arid Australia can breed continuously under favorable conditions. During droughts, however, breeding ceases but is said to begin again immediately after rain. We found that testis size in breeding birds did not change during, or between, reproductive cycles. Luteinizing hormone (irLH) levels in plasma, however, were significantly higher in males during early incubation (1.25 *ng*/ml), and in females during courtship (0.89 *ng*/ml), than during other parts of the reproductive cycle. Plasma levels of the sex steroids were highest (1.06 *ng*/ml for androgen in males, 0.32 *ng*/ml for estrogens in females) at the same time that irLH levels were highest. Wild-caught Zebra Finches had, at capture, testes similar in size to those of aviary-breeding Zebra Finches, but after three weeks of dehydration (1 ml water/bird/week), testis size was significantly smaller and hematocrit was significantly higher: 54% vs. 50% for birds given unlimited access to water. When dehydrated birds were given unlimited access to water, testes grew significantly. Access to green grass or exposure to high relative humidity (85%) augmented the effects of the water on testis size. Spermatogenic activity in some dehydrated birds was high despite small gonad size; however, interstitial tissue was poorly developed, and plasma levels of sex steroids and irLH were low. We suggest that Zebra Finches normally maintain gonadal tissue in a functioning state unless severely dehydrated. Relief from dehydration is a necessary, but not sufficient, condition for the full expression of reproduction.

For species that do not live in temporally uniform environments, the timing of reproduction can be a critical element in reproductive success. In many temperate zone birds, reproductive activity begins during the spring, when increasing daylength provides a dependable cue that indicates the approach of conditions favorable for reproduction (see reviews in Lofts and Murton 1968, Farner 1970, Farner and Lewis 1971, Wingfield and Farner 1980). Compared to daylength, other environmental cues that may play a role in controlling the timing of reproduction have received little attention (Moreau 1950, Marshall and Disney 1957, Marshall 1970, Immelmann 1971, Wingfield 1980, Earle 1981, Storey and Nicholls 1982). In some habitats, daylength may not be a reliable cue for reproduction because the onset of other favorable conditions is independent of the changes in daylength. Desert birds, for example, may encounter suitable conditions for reproduction only for brief periods after rainfall, and in such species rapid initiation of reproduction in response to rainfall, regardless of daylength or season, may maximize reproductive success.

Such breeding in response to rain apparently occurs in the Zebra Finch (*Poephila guttata*, syn: *Taeniopygia guttata castanotis*) native to the arid interior of Australia. The reproductive state of Zebra Finches is not influenced by daylength (Marshall and Serventy 1958, Oksche et al. 1963, Sossinka 1975). In the mesic parts of their range, or in areas receiving agricultural irrigation, Zebra Finches breed year-round, except in the coldest winter months, with peaks in nesting attempts occurring in spring and in autumn (Frith and Tilt 1959, Kikkawa 1980). In arid central Australia, however, the species breeds following the irregular rains, regardless of the time of year (Immelmann 1965). Davies (1977), in a two-year study of Zebra Finches in Western Australia, found birds breeding in every month except March, April, and August, and suggested that both rainfall and temperature were important in the initiation of breeding. Immelmann (1963, 1965) reported that Zebra Finches copulated within a few hours of the beginning of the first rain following several months of drought, and began nesting and egg-laying within one week. Zebra Finches are capable of producing many offspring under favorable conditions because (1) both members of the pair participate in nest building (Immelmann 1963), (2) a pair will nest repeatedly as long as conditions remain favorable (Ser-

venty 1971), and (3) they become sexually mature at an early age, even under sub-optimal conditions (Sossinka 1972, 1975, 1980).

Possible factors that trigger reproduction in Zebra Finches and other xerophilic birds include: temperature (Serventy and Marshall 1957, Frith and Tilt 1959, Davies 1977, Kikkawa 1980); nutritional state of the female (Perrins 1970, Jones and Ward 1976); green grass or other materials used for nest-building (Marshall and Disney 1957, Serventy 1971); social or psychological factors such as sight of rain, clouds, or other courting birds (Immelmann 1963, Serventy 1971); and the effects of rain (reviewed in Immelmann 1971, Serventy 1971). The effects of rain may act either directly by providing drinking water or breeding sites (Frith 1967) or indirectly by increasing humidity (Priedkalns and Bennett 1978) or increasing food supply (Immelmann 1970). Kikkawa (1980) reviewed factors thought to set off breeding in Zebra Finches; he suggested that more than one factor may be involved and that the relative importance of these factors may vary in different parts of the species' range.

Neither the hormonal control of reproduction in rain-breeding birds nor the mechanisms by which rain or its effects trigger the beginning of reproduction have been clearly determined. Farner and Serventy (1960) suggested that Zebra Finches may have a tonic production of the pituitary gonadotropins that stimulate gonadal development. This constant secretion might be inhibited only by specific external factors such as extreme temperature and lack of water. Therefore, under most circumstances, the birds would be reproductively ready or nearly so, and could respond immediately to favorable conditions such as those following rains.

We report here how gonad size, plasma levels of the gonadotropin, luteinizing hormone (irLH), and the sex steroids change during a reproductive cycle in captive Zebra Finches and compare these to cycles in photoperiodic species of birds. We also discuss how the availability of free water, presence of green grass, and humidity affect gonad size and hormone levels in wild-caught Zebra Finches.

MATERIALS AND METHODS

AVIARY BIRDS

We obtained adult Zebra Finches from commercial suppliers in Adelaide, South Australia. Between January and June 1980, 13–24 pairs of birds were kept in two outdoor aviaries (~3 × 4 m, 5–7 pairs at a time; and ~1.5 × 2.5 m, 4–5 pairs at a time) where they had access to nest boxes, nesting material, finch seed, water, and grit. All birds were marked with colored leg bands so that individuals could be identified. We observed the behavior of the birds at least once a week for 1–3 h and recorded which birds were courting and the stage of the nesting cycles for each mated pair. At two-week intervals, we took blood samples, for hormone analysis, from courting or breeding pairs. Each male was laparotomized in order to measure the length of the left testis. A blood sample of approximately 250 μl was then taken from the basilic vein in the wing. Birds had been removed from the aviary for 15 min to 2 h before blood samples were taken. The blood was centrifuged, the hematocrit measured, and the plasma stored at −20°C for later hormone analysis. In most cases, these procedures had no obvious effect on the bird's subsequent behavior.

WILD-CAUGHT BIRDS

We captured 16 male and 16 female Zebra Finches near Barmera, South Australia (34°S) on 26 February and 10 March 1980. The birds at this site had access to water and some of the birds we caught may have been breeding. The birds were placed in a controlled-environment room at 27 ± 2°C, illuminated by both fluorescent and incandescent light on a 14:10 (light : dark) daily photocycle. The relative humidity, measured with a recording hygrothermograph, averaged 40% (=11 torr water vapor pressure), and ranged from 25–60% except during high humidity treatments (see below). Birds were kept in male-female pairs in small cages (60 × 45 × 30 cm), within sight and hearing range of other pairs. Eight of the males were laparotomized and blood samples were taken (as described above for aviary birds) the day after capture; the other eight males were laparotomized two weeks after capture.

Following their arrival in the laboratory, we gave the birds unlimited quantities of finch seed (water content 11–12%) and access to water for at least nine days, after which freely available water was gradually withdrawn over the course of twelve days until they were receiving only 1 ml/bird/week (dehydration conditions). This intake of water represents 2–6% of their normal unlimited water intake (Oksche et al. 1963, Calder 1964, Cade et al. 1965, Lee and Schmidt-Nielsen 1971, Skadhauge and Bradshaw 1974). The water contained a liquid vitamin supplement (ABDEC Baby Vitamin). After two weeks of dehydration, the 16 pairs were divided into four groups that received one of the following experimental treatments: (1) access to unlimited volumes of water, (2) access to freshly picked green grass, but only

1 ml water/week, (3) access to both unlimited amounts of water and green grass, or (4) continuation of the dehydration conditions of 1 ml water/week. Before the experimental treatments began, and after three weeks of treatment, each bird was laparotomized and a blood sample was taken, as described above. It took from 10 to 20 min handling time to laparotomize and bleed each bird.

Following this first experiment, we again subjected all birds to the dehydration conditions (1 ml water/week and no green grass) for three weeks. At the end of this second dehydration period, the humidity in the controlled-environment chamber was increased by placing a humidifier in the chamber. The relative humidity in the chamber increased to an average of 85% (=23 torr water vapor pressure) and ranged from about 60 to 95%. At this high humidity, the birds were given the same experimental treatments as described above, i.e., access to water, green grass, both, or neither. As before, the birds were laparotomized and blood samples were taken immediately before, and three weeks after, the start of this experimental treatment.

At the end of the second experimental treatment, we killed nine of the males (selected from among the four treatment groups), by decapitation and removed their testes. Testes were immediately weighed to the nearest mg and were preserved in buffered formalin for histological examination. The formation and proliferation of spermatozoa in seminiferous tubules were rated, using a range of 1 (no spermatogenesis) to 5 (maximum spermatogenesis) comparable to the 1–7 range of Blanchard (1941).

HORMONE ASSAYS

We measured, by radioimmunoassay, the plasma levels of the sex steroids (androgens for male, estrogen for female blood samples) and irLH. Because plasma levels of the steroids were generally low and because only small amounts of blood could be obtained from individual birds, most individual samples would have been below the detectable limits of the steroid assays. Consequently, plasma samples within treatment groups were pooled for the steroid assays. This assay is described in detail by Kerin et al. (1981). The androgen antiserum was raised against testosterone-3-(O-carboxymethyl) oxime conjugated to bovine serum albumin and used at a dilution of 1:20,000. The cross-reactivity of the testosterone antiserum was as follows: testosterone, 100%; androstenedione, 1.3%; 5α-dihydrotestosterone, 31%; 4-androsten-3β-17β-diol, 30%; and 4-androsten-17β, 19-diol-3-one, 3.5%. The estrogen antiserum was raised against estrogen-6-(O-carboxymethyl) oxime conjugated to bovine serum albumin and used in a dilution of 1:16,000. The cross reactivity values of the estrogen antiserum, were as follows: estradiol-17β, 100%; estrone, 17%; estrol, 0.6%; testosterone, 0.2%; and progesterone, less than 0.1%. The limits of assay sensitivity for estrogen and androgen were 10 pg/ml of plasma. The intra- and interassay coefficients of variation for all assays were less than 10% and 20%, respectively (Seamark, pers. comm.).

Plasma levels of irLH were measured in the laboratory of D. S. Farner (University of Washington, Seattle) using a double-antibody radioimmunoassay for chicken LH. The radioimmunoassay was described in detail by Follett et al. (1972) and was modified for use with small birds by Follett et al. (1975). Serial dilutions of Zebra Finch blood respond in parallel with purified chicken LH to the antibody, indicating specificity of the antibody to immunoreactive Zebra Finch LH (Wingfield, unpubl. data).

STATISTICS

Numerical results are reported as means ± SD with sample size (n) in parentheses. Means are compared using two-tailed Student's t-tests. For comparisons between behavioral categories (see below), one-way analysis of variance (ANOVA) and Newman-Keuls multiple range tests (Zar 1974) were used to assess statistical significance. Two-way ANOVA was used to analyze results of the laboratory experiments on wild-caught birds. In testing hypotheses, P-values less than 0.05 were considered to be significant.

RESULTS

AVIARY BIRDS

The plasma levels of androgens we report here (Table 1) are similar to values for testosterone titer reported by Sossinka et al. (1980), Luine et al. (1980), and Pröve and Immelmann (1982), and are lower than the highest values reported by Pröve (1978) for actively courting Zebra Finches. Our lower values may be the result of decreases in hormone levels during the time we took to capture the birds and take blood samples, since the stress associated with handling is known to decrease hormone titer in Zebra Finches (Pröve 1978, pers. comm.).

Data from aviary birds were divided into six categories, depending on the birds' behavior: (1) birds that were actively courting or being courted, but which did not successfully nest during the following week, (2) courting birds that nested within the following week, (3) birds

TABLE 1. Testis length and reproductive hormone levels in Zebra Finches breeding in aviaries or caught in the field and kept in small cages with only limited water.[a]

	Left testis length	irLH ng/ml ± SD (n)		Male plasma androgens	Female plasma estrogens
	mm ± SD (n)	Male	Female	ng/ml (n)[b]	ng/ml (n)[b]
Aviary birds					
Unsuccessful courtship	4.1 ± 0.6 (5)	0.94 ± 0.25 (9)	0.41 ± 0.18 (10)	0.50 (8)	<0.13 (5)
Successful courtship	3.8 ± 0.8 (8)	1.04 ± 0.38 (10)	0.89 ± 0.50 (9)	0.80 (9)	0.32 (8)
Early incubation	4.1 ± 0.6 (7)	1.24 ± 0.83 (7)	0.70 ± 0.14 (4)	1.06 (4)	0.18 (5)
Late incubation	3.9 ± 0.1 (4)	0.63 ± 0.41 (7)	0.38 ± 0.28 (7)	0.24 (5)	<0.16 (4)
Feeding nestlings	3.8 ± 0.7 (4)	0.49 ± 0.25 (6)	0.79 ± 0.28 (4)	0.25 (4)	0.45 (3)
Feeding fledglings	4.0 ± 0.7 (2)	1.12 (1)	0.77 ± 0.21 (2)	–	–
Field-caught birds					
At capture	3.5 ± 1.0 (16)	0.45 ± 0.25 (15)	0.47 ± 0.42 (14)	0.30 (5)	0.19 (16)
After first dehydration	2.7 ± 0.7 (16)	0.41 ± 0.23 (12)	0.42 ± 0.26 (12)	<0.28 (16)	<0.24 (16)
After second dehydration	3.0 ± 0.6 (16)	0.55 ± 0.28 (16)	0.59 ± 0.36 (13)	<0.23 (16)	<0.13 (16)

[a] Birds were given one ml water/week.
[b] For the sex steroids, the values are from pooled samples of blood and the sample size indicates the number of birds whose blood was added to the pool.
[c] Brackets connect values that are statistically significantly different ($P < 0.05$).

in the first week of incubation, during which time the clutch was not complete for some pairs, (4) birds that were in the second week of incubation, (5) birds that were feeding nestlings, and (6) birds that were feeding fledglings. For birds in the first two categories, we used only individuals that courted each other exclusively during the previous 1–3 h observation period. The nestling period lasts about three weeks and the fledgling period an additional two weeks. Both members of the pair incubate and feed the young (pers. observ.).

Testis length did not vary between behavioral categories (one-way ANOVA, $F = 0.35$, $P > 0.75$). In addition, testis length in breeding birds did not differ from that in non-breeding birds at the beginning of the experiment when they had just been released into the aviary (3.9 ± 0.5 mm, $n = 14$).

Hormone levels, however, did vary significantly between behavioral categories (Table 1). The levels of irLH were significantly higher in males during early incubation than in males feeding nestlings. The levels of irLH in females were significantly higher during successful courtship than during late incubation or during unsuccessful courtship when the birds did not subsequently nest. Levels of gonadotropin in both sexes began to increase again during the last stages of the reproductive cycle, presumably in preparation for the next brood. Females will often begin a second clutch while the male is still feeding the young from the first clutch (pers. observ.).

The levels of plasma estrogen in females remained relatively low throughout the reproductive cycle. They were highest during courtship and at the end of the reproductive cycle. The levels of androgens in males were highest during courtship and early incubation coincident with peak irLH values (Table 1). Because the values for steroids were from blood samples pooled from several individuals, no statistical treatment was undertaken.

WILD-CAUGHT BIRDS

Wild-caught Zebra Finches were significantly smaller (11.0 ± 0.8 g, $n = 32$) than the commercially obtained birds (12.0 ± 0.8 g, $n = 22$). These values are slightly lower than the body masses of wild and domesticated Zebra Finches reported by Sossinka (1970). Body masses of the wild-caught Zebra Finches did not change with dehydration. Body mass did not differ significantly between sexes in either wild-caught or commercially-obtained birds.

When the wild-caught Zebra Finches were brought in from the field, their mean testis length was not significantly different from that of Zebra Finches breeding in outdoor aviaries (Table 1). The level of irLH in males measured the day after capture (0.60 ± 0.18 ng/ml, $n = 8$) was significantly higher than that measured in males measured two weeks after capture (0.29 ± 0.23 ng/ml, $n = 7$), but the levels of androgen in the two groups were indistinguishable. Plasma levels of irLH in recently caught males did not differ from those in the non-breeding aviary males (0.73 ± 0.31 ng/ml, $n = 7$), but were significantly lower than those of successfully courting male Zebra Finches in the aviaries (Table 1).

In the wild-caught males, mean testis length decreased significantly ($t = 4.78$, $P < 0.01$) during the first dehydration period. Mean testis length after the second dehydration period was not significantly different from that after the first dehydration treatment (Table 1). The level of irLH did not differ between values measured just after capture and those measured after each of the dehydration treatments.

The protocol for the wild-caught birds gen-

TABLE 2. Plasma level of irLH (*n*g/ml) in dehydrated Zebra Finches before and after three weeks exposure to the indicated experimental conditions (see Materials and Methods). Values given are means ± SD. There were four birds in each group.

		Water			
		Ad libitum		1 ml/wk	
		Before	After	Before	After
Low humidity	Grass	0.39 ± 0.26	0.21 ± 0.14	0.22 ± 0.17	0.26 ± 0.27
	No grass	0.44 ± 0.37	0.51 ± 0.18	0.49 ± 0.10	0.23 ± 0.26
High humidity	Grass	0.49 ± 0.29	0.52 ± 0.18	0.71 ± 0.41	0.60 ± 0.20
	No grass	0.55 ± 0.37	0.44 ± 0.03	0.55 ± 0.13	0.17 ± 0.14

TABLE 3. Left testis length (mm) in dehydrated Zebra Finches before and after three weeks of exposure to the indicated experimental conditions (see Materials and Methods). Values given are means ± SD. There were four birds in each group.

		Water			
		Ad libitum		1 ml/wk	
		Before	After	Before	After
Low humidity	Grass	2.9 ± 0.9	3.3 ± 0.5	2.8 ± 0.6	2.9 ± 0.7
	No grass	2.0 ± 0.4	3.5 ± 0.9	3.1 ± 0.6	2.9 ± 0.9
High humidity	Grass	3.3 ± 0.5	3.5 ± 0.4	2.9 ± 0.6	3.3 ± 0.9
	No grass	3.0 ± 0.7	3.4 ± 0.6	2.9 ± 0.9	3.1 ± 0.6

erated eight experimental groups. After two or three weeks under dehydration conditions (see Methods), we gave each group one of eight different combinations of water (unlimited or restricted volumes), green grass (access or no access), and humidity (exposure to 40% or 85% relative humidity). The plasma levels of irLH in the males did not change significantly in any of the eight experimental groups (Table 2). In none of the eight groups were androgen levels high enough to measure with the assay we used.

Access to unlimited volumes of water had a significant positive effect on testis size (Tables 3 and 4). Neither green grass nor humidity by itself affected testis size. There were, however, significant interaction effects of grass plus water, and high humidity plus water. That is, when unlimited volumes of water were available, the additional presence of green grass or high humidity resulted in further increases in testis size. There was no further significant interaction when all three factors were considered together. These results indicate that relieving the stress of dehydration by adding freely available water to the diet has a direct positive effect on testicular size in Zebra Finches, and that this positive effect is augmented by the presence of green grass or an increase in relative humidity.

TABLE 4. Analysis of variance of change in testis length in individual Zebra Finches exposed to different conditions after dehydration.

Source of variation	df	Mean squares	*F*-ratio	*P*
Main effects				
Water	1	2.00	8.00	0.009
Grass	1	0.28	1.13	0.299
Humidity	1	0.13	0.50	0.486
Interaction effects				
Water and grass	1	1.53	6.13	0.021
Water and humidity	1	2.00	8.00	0.009
Grass and humidity	1	0.28	1.13	0.299
Water, grass, and humidity	1	0.78	3.13	0.090
Constant	1	4.50	18.0	0.000
Error	24	0.25	—	—

Measurement of hematocrit provides some insight into how our dehydration treatments affected water balance in the Zebra Finches (Table 5). Hematocrit is known to increase with water stress in other birds (Koike et al. 1983). Hematocrits were significantly higher in birds given only 1 ml water/week than in birds given unrestricted access to water, but access to green grass or exposure to high or low humidity had no effect on hematocrits (Table 6). The hematocrits of wild-caught Zebra Finches given free access to water (Table 5) were not significantly different from the hematocrits of finches breeding in the aviary (0.50 ± 0.04, $n = 24$), whereas the hematocrits of the wild-caught Zebra Finches restricted to 1 ml water/week were significantly higher than the hematocrits of aviary birds.

Testis size was not correlated with spermatogenic activity, judged by histological examination in the nine birds we sampled (Fig. 1). The Spearman Rank correlation coefficient between the two was 0.406 ($Z = 1.22$, $P >$

TABLE 5. Hematocrit in male Zebra Finches exposed to eight different conditions (free or limited access to water and/or green grass and exposure to low or high humidity). Values given are means ± SD, sample size in parentheses.

		Water	
		Ad libitum	1 ml/wk
Low humidity	Grass	0.53 ± 0.03 (5)	0.54 (1)
	No grass	—	0.54 ± 0.04 (19)
High humidity	Grass	0.50 ± 0.02 (9)	0.55 ± 0.04 (4)
	No grass	0.51 ± 0.02 (4)	0.53 ± 0.07 (8)

TABLE 6. Analysis of variance of hematocrits in male Zebra Finches exposed to different conditions.

Source of variation	df	Mean squares	*F*-ratio	*P*
Main effects				
Water	1	102.72	6.15	0.017
Grass	1	0.65	0.04	0.845
Humidity	1	9.78	0.58	0.449
Interaction effects				
Water and grass	1	9.30	0.56	0.460
Water and humidity	1	11.38	0.68	0.414
Grass and humidity	1	1.47	0.09	0.768
Water, grass, and humidity	0	—	—	—
Constant	1	139,392	8339	0
Error	43	16.71	—	—

0.20). Index of spermatogenic activity was also not related to treatment group. Despite spermatogenic activity within the seminiferous tubules in some testes, we found no indication of activity in the interstitial tissue of any of the testes examined (Fig. 2).

DISCUSSION

The reproductive biology of Zebra Finches is best understood in terms of its adaptive significance to the arid environment in which the birds live. From the viewpoint of avian reproduction, one important feature of the Australian deserts is their unpredictable rainfall pattern (Immelmann 1970). Favorable conditions for avian reproduction do not occur on a regular seasonal basis, but follow irregular rains that may occur at any time of the year. Thus, photoperiod is of little value to Zebra Finches as a cue for reproduction. In the interior of Australia and in similar environments elsewhere, natural selection is likely to have set a premium on a bird's ability to respond rapidly to suitable reproductive conditions whenever they become available.

REPRODUCTIVE CYCLES IN ZEBRA FINCHES

The tremendous temporal variation in gonad size that occurs in many temperate-zone birds is absent in Zebra Finches. In our study, testis length did not change during a reproductive cycle (from courtship through fledging of chicks), and, under aviary conditions, the testes did not regress between clutches (Table 1). Testis length did decline significantly when birds were dehydrated, but even then only by about 20%. In free-living birds, testis length usually

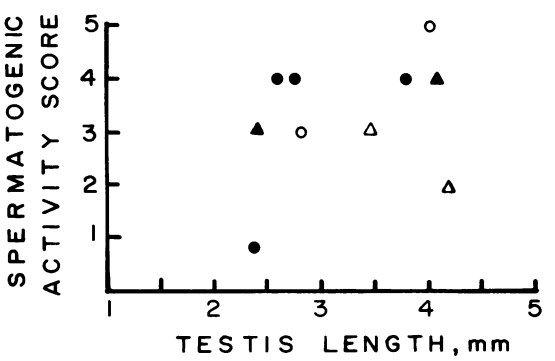

FIGURE 1. Relation between spermatogenic activity and testis length in Zebra Finches. Solid circles represent birds not given free access to green grass or water; open circles, birds given access to grass but not water; solid triangles, birds given access to unlimited volumes of water but not quantities of grass; and open triangles, birds given free access to both water and green grass. All birds were maintained at 27°C and a relative humidity of 85% before dissection.

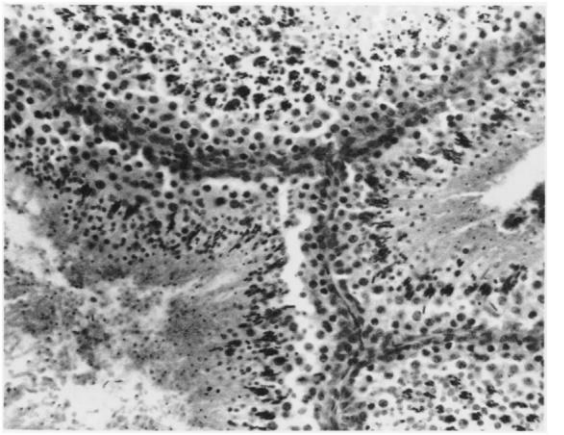

FIGURE 2. Photomicrograph (×104) of seminiferous tubules from a Zebra Finch. Note active spermatogenesis in seminiferous tubules (spermatogenic score = 4) and sparse interstitial (Leydig gland) tissue. This bird was kept at 85% relative humidity, but had only 1 ml water/week and no access to green grass for 3 weeks before dissection.

remains between 3 and 4 mm year-round, just as in our aviary birds, and while decreases in testis length of 20–40% do occur in wild birds, they do not occur at regular intervals, but appear to be associated with periods of drought (Davies 1977).

Other researchers (Oksche et al. 1963, Sossinka 1974) have not been able to demonstrate an effect of dehydration on gonad size in Zebra Finches. This may be due to a difference in technique. While we used serial laparotomies to monitor change in gonad size within individuals, previous studies compared gonad sizes between birds given free access to water and those without water. This latter method is less able to detect small changes within individuals against a background of substantial variation between individuals.

In contrast to the situation in Zebra Finches, in birds that breed in response to increasing daylength, gonads regress after spring or summer breeding and remain small through the winter; then, as daylength increases in the spring, gonad sizes increase logarithmically (Farner and Gwinner 1980, Wingfield and Farner 1980). For example, in one race of the White-crowned Sparrow (*Zonotrichia leucophrys pugetensis*), testis mass increases from only 1–3 mg in the winter to nearly 500 mg just before breeding in the spring. These sparrows typically lay two clutches each year, and testis mass declines after the first clutch, then increases again for the second clutch (Wingfield and Farner 1978).

Our finding that gonads of Zebra Finches do not change size during or between reproductive cycles except when the birds are severely water-stressed, supports Farner and Serventy's (1960) hypothesis regarding the tonic maintenance of gonadal tissue. By maintaining gonads in a near-breeding condition, the birds are able to respond almost immediately to the unpredictable onset of conditions favorable for reproduction. In contrast, wild populations of White-crowned Sparrows take over two months to achieve full gonadal recrudescence (King et al. 1966). In a species for which conditions favorable for reproduction are predictably seasonal, it is apparently adaptive for the birds to re-allocate energy reserves from gonadal maintenance to other functions during non-reproductive seasons, and to re-grow gonadal tissue in anticipation of the return of favorable conditions. For arid-zone birds, gonadal regression and gradual recrudescence may not be a viable option. If a Zebra Finch of arid Australia required two months to reach reproductive readiness following rain, it might completely miss suitable conditions for breeding!

Levels of the gonadotropin, irLH, and the sex steroids do change during a reproductive cycle in Zebra Finches in a pattern similar to that found in other birds, although the amplitude of variation in the plasma levels of these hormones is not as great as is seen in some other birds (Wingfield and Farner 1980). In White-crowned Sparrows, plasma levels of testosterone and irLH increase about ten-fold over the course of the year, and, like testis mass, male irLH and androgen levels decrease between clutches during the breeding season (Wingfield and Farner 1978). Sexual behavior in the male Zebra Finch is dependent on androgen levels (Pröve 1974, 1978; Arnold 1975; Harding et al. 1983). The birds' behavior changes during a breeding cycle, and therefore it is not surprising that hormones controlling these behaviors also change.

WATER AND REPRODUCTION IN ZEBRA FINCHES

In arid parts of Australia, Zebra Finches are most abundant near permanent bodies of water, but they are capable of surviving without access to drinking water, providing temperatures are moderate (Oksche et al. 1963; Calder 1964; Cade et al. 1965; Lee and Schmidt-Nielsen 1971; Sossinka 1972, 1974). Certainly, wild Zebra Finches may not always have daily access to water. In many Australian birds, breeding activity declines during drought and increases following rains, regardless of the time of year. (Keast and Marshall 1954; Serventy and Marshall 1957; Keast 1959, 1968; Frith 1967). Thus it seems likely that water stress may inhibit reproduction, and access to water and subsequent rehydration may release this inhibition. Of course, even when water is abundant, other factors, such as extreme temperature, may still prevent breeding.

We have demonstrated that reduction in water availability leads to dehydration of the birds as evidenced by the significant increase in hematocrit, and that dehydration is associated with a significant decrease in testis length in male Zebra Finches. In our experiments, the presence of unlimited water relieved the effects of dehydration and had a direct positive effect on testicular size within individuals. Even small increases in testis length represent a significant increase in the volume of testicular tissue, because the volume increases roughly with the cube of a linear dimension. The positive effect of water on testis length was augmented by the presence of green grass or an increase in relative humidity. The ingestion of green grass adds free water to the bird's diet and increased relative humidity decreases the bird's pulmonary evaporative water loss, so both of these

factors further ameliorate the effects of dehydration.

It is possible that the green grass stimulates reproductive readiness because it provides a chemical signal that acts as a reproductive trigger, as has been demonstrated in microtine rodents (Negus and Berger 1977, Berger et al. 1981, Sanders et al. 1981). To our knowledge, however, there is no direct evidence for similar effects in birds. Alternatively, the grass could stimulate the reproductive system because it is the material used in nest building. Lehrman (1973) demonstrated that pairs of Ringed Turtle-Doves (*Streptopelia risoria*) that had been kept together in small cages began to build nests immediately when given nesting material. We did not see, however, the Zebra Finches in our experiments manipulate the grass as they do nest material, whereas they always ate green grass when it was available.

The plasma levels of irLH and androgen in all the experimental birds were similar to those of non-breeding aviary birds, lower than those of courting birds, and did not increase with the addition of water. This result is not surprising in view of the fact that the experimental birds were in small cages without access to nests (see Pröve 1978). There are undoubtedly subsidiary factors including physical, physiological, and social conditions that are important in fine-tuning reproduction (Wingfield 1980, 1983). For example, Moore (1983) demonstrated that reproductive function of male White-crowned Sparrows was profoundly affected by the endocrine state and behavior of the female. Relief from dehydration may be a necessary, but not a sufficient, condition for the full expression of reproductive physiology and behavior.

HORMONAL CONTROL OF REPRODUCTION IN ZEBRA FINCHES

In seasonally breeding birds, the testes are generally gametogenetically inactive in their winter, regressed condition (Lofts and Murton 1973). The histological condition of the regressed testis is usually completely uniform, the germinal epithelium of the collapsed seminiferous tubule consisting only of a single layer of spermatogonia along with Sertoli cells. High levels of spermatogenic activity are usually found only in enlarged testes (Blanchard 1941). Thus, it is surprising that spermatogenic activity in the Zebra Finch is neither closely related to season or testes size, nor related to their state of hydration. We found some relatively large testes with inactive seminiferous tubules, and some relatively small testes with mature sperm within the lumen of seminiferous tubules. Some birds, not given access to water, evidenced higher levels of spermatogenic activity than other birds with access to water (Fig. 1). Oksche et al. (1963) also found Zebra Finches that were subjected to restricted water intake continued to produce spermatozoa. Keast and Marshall (1954), however, reported that at a desert site where little rain had fallen for three years, gonads of Zebra Finches were small and inactive.

It appears that in the Zebra Finch, even when the testes are reduced in size because of severe dehydration, some spermatogenesis can occur. Tonic low level of spermatogenesis is obviously of potential adaptive value to males that must be ready for the immediate copulations that occur with rains following severe drought (Immelmann 1963). The total numbers of sperm produced by such birds, however, is probably low relative to those of non-water-stressed birds with larger testes.

The poorly developed interstitial tissue of the testes of all the experimental birds correlates well with the low levels of plasma irLH and androgens in these birds (Nicholls and Graham 1972, Lam and Farner 1976). In laboratory strains of Common Quail (*Coturnix coturnix*) pituitary LH stimulates activity in the steroidogenic interstitial cells of Leydig (Brown et al. 1975). The androgens then control the development of sexual accessory organs, secondary sexual characteristics such as courtship, and possibly gamete production of the seminiferous tubules (see review in Lofts and Massa 1980). It remains to be explained why gonad size in dehydrated Zebra Finches increases with access to water, while plasma levels of irLH and androgens levels remain low. One hypothesis is that plasma levels of follicle-stimulating hormone (FSH) may decrease with dehydration and increase with relief from water stress, and that the level of FSH controls changes in gonad size. FSH is known to cause testicular growth in Common Quail (Brown et al. 1975, Follett 1980) and is thought to increase spermatogenic activity of the seminiferous tubules by stimulating local androgen secretion of Sertoli cells, which in turn influences production of adjacent germ cells (Lofts and Massa 1980). LH and subsequently plasma androgens may increase only when other conditions for reproduction, such as presence of mates and nests, are suitable.

ACKNOWLEDGMENTS

This work was supported in large part by the University of Adelaide and in small part by the first author. We are deeply indebted to D. S. Farner (University of Washington) and R. Seamark (University of Adelaide), in whose laboratories the radioimmunoassays were done. Leila Mak (Queen Elizabeth Hospital, Adelaide) performed the steroid assays. John Wingfield, Marilyn Ramenofsky, Phil Mattocks, and G. J. Kenagy aided with the LH assays.

Roger Seymour, Shelley Barker, and the Department of Zoology of the University of Adelaide loaned space and equipment. David Vleck read each draft of this paper, and Joel Brown and Anne Moffett provided further assistance.

LITERATURE CITED

ARNOLD, A. P. 1975. The effects of castration and androgen replacement on song, courtship, and aggression in Zebra Finches (*Poephila guttata*). J. Exp. Zool. 191:309–326.

BLANCHARD, B. D. 1941. The White-crowned Sparrows (*Zonotrichia leucophrys*) of the Pacific seaboard: environment and annual cycle. Univ. Calif. Publ. Zool. 46:1–177.

BROWN, N. L., J. D. BÁYLE, C. G. SCANES, AND B. K. FOLLETT. 1975. Chicken gonadotrophins: their effects on the testes of immature and hypophysectomized Japanese Quail. Cell Tissue Res. 156:499–520.

BERGER, P. J., N. C. NEGUS, E. H. SANDERS, AND P. D. GARDNER. 1981. Chemical triggering of reproduction in *Microtus montanus*. Science 214:69–70.

CADE, T. J., C. A. TOBIN, AND A. GOLD. 1965. Water economy and metabolism of two estrildine finches. Physiol. Zool. 38:9–33.

CALDER, W. A. 1964. Gaseous metabolism and water relations of the Zebra Finch, *Taeniopygia castanotis*. Physiol. Zool. 37:400–413.

DAVIES, S. J. J. F. 1977. The timing of breeding by the Zebra Finch *Taeniopygia castanotis* at Mileura, Western Australia. Ibis 119:369–372.

EARLE, R. A. 1981. Factors governing avian breeding in *Acacia* savanna, Pietermaritzburg, Part 1; extrinsic factors. Ostrich 52:67–73.

FARNER, D. S. 1970. Day length as environmental information in the control of reproduction of birds, p. 71–88. *In* J. Benoit and I. Assenmacher [eds.], La photorégulation de la reproduction chéz les oiseaux et les mammifères. Cent. Natl. Rech. Sci., Paris.

FARNER, D. S., AND E. GWINNER. 1980. Photoperiodicity, circannual and reproductive cycles, p. 331–336. *In* A. Epple and M. H. Stetson [eds.], Avian endocrinology. Academic Press, New York.

FARNER, D. S., AND R. A. LEWIS. 1971. Photoperiodism and reproductive cycles in birds. Photophysiology 6:325–370.

FARNER, D. S., AND D. L. SERVENTY. 1960. The timing of reproduction in birds in the arid regions of Australia. Anat. Rec. 137:354.

FOLLETT, B. K. 1980. Gonadotrophin secretion in seasonally breeding birds and its control by daylength. Proc. XVII Int. Ornithol. Congr. (1978):239–244.

FOLLETT, B. K., C. G. SCANES, AND F. J. CUNNINGHAM. 1972. A radioimmunoassay for avian luteinizing hormone. J. Endocrinol. 52:359–378.

FOLLETT, B. K., D. S. FARNER, AND P. W. MATTOCKS. 1975. Luteinizing hormone in the plasma of White-crowned Sparrows, *Zonotrichia leucophrys gambelii*, during artificial photostimulation. Gen. Comp. Endocrinol. 26:126–134.

FRITH, H. J. 1967. Waterfowl in Australia. East-West Center Press, Honolulu, HI.

FRITH, H. J., AND R. A. TILT. 1959. Breeding of the Zebra Finch in the Murrumbidgee Irrigation Area, New South Wales. Emu 59:289–295.

HARDING, C. F., K. SHERIDAN, AND M. J. WATERS. 1983. Hormonal specificity and activation of sexual behavior in male Zebra Finches. Horm. Behav. 17:111–133.

IMMELMANN, K. 1963. Drought adaptations in Australian desert birds. Proc. XIII Int. Ornithol. Cong. (1962): 649–657.

IMMELMANN, K. 1965. Australian finches. Angus and Robertson, Sydney, Australia.

IMMELMANN, K. 1970. Environmental factors controlling reproduction in African and Australian birds—a comparison. Ostrich Suppl. 8:193–204.

IMMELMANN, K. 1971. Ecological aspects of periodic reproduction, p. 341–389. *In* D. S. Farner and J. R. King [eds.], Avian biology. Vol. I. Academic Press, New York.

JONES, P. J., AND P. WARD. 1976. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the Red-billed Quelea, *Quelea quelea*. Ibis 118:547–574.

KEAST, A. 1959. Australian birds: their zoogeography and adaptations to an arid environment, p. 89–114. *In* A. Keast, R. L. Crocker, and C. S. Christian [eds.], Biogeography and ecology in Australia. Junk, The Hague.

KEAST, A. 1968. Moult in birds of the Australian dry country relative to rainfall and breeding. J. Zool. (Lond.) 155:185–200.

KEAST, A., AND A. J. MARSHALL. 1954. The influence of drought and rainfall on reproduction in Australian desert birds. Proc. Zool. Soc. Lond. 124:493–499.

KERIN, J. F., T. J. BROOM, M. M. RALPH, D. K. EDMONDS, G. M. WARNERS, R. JEFFREY, J. M. CROCKER, B. GODFREY, L. W. COX, R. F. SEAMARK, AND C. D. MATHEWS. 1981. Human luteal phase function following oocyte aspiration from the immediate preovular graafian follicle of spontaneous ovular cycles. Br. J. Obstet. Gynaecol. 88:1021–1028.

KIKKAWA, J. 1980. Seasonality of nesting by Zebra Finches at Armidale, New South Wales. Emu 80:13–20.

KING, J. R., B. K. FOLLETT, D. S. FARNER, AND M. M. MORTON. 1966. Annual gonadal cycles and pituitary gonadotropins in *Zonotrichia leucophrys gambelii*. Condor 68:476–487.

KOIKE, T. I., L. R. PRYOR, AND H. L. NELDON. 1983. Plasma volume and electrolytes during progressive water deprivation in chickens (*Gallus domesticus*). Comp. Biochem. Physiol. A Comp. Physiol. 74:83–87.

LAM, F., AND D. S. FARNER. 1976. The ultrastructure of the cells of Leydig in the White-crowned Sparrow (*Zonotrichia leucophrys gambelii*) in relation to plasma levels of luteinizing hormone and testosterone. Cell Tissue Res. 169:93–109.

LEE, P., AND K. SCHMIDT-NIELSEN. 1971. Respiratory and cutaneous evaporation in the Zebra Finch: effect on water balance. Am. J. Physiol. 220:1598–1605.

LEHRMAN, D. S. 1973. Hormonal regulation of parental behavior in birds and infrahuman mammals, p. 1268–1382. *In* W. C. Young [ed.], Sex and internal secretions. Vol. 2. Krieger Publ. Co., Huntington, NY.

LOFTS, B., AND R. MASSA. 1980. Male reproduction, p. 413–434. *In* A. Epple and M. H. Stetson [ed.], Avian endocrinology. Academic Press, New York.

LOFTS, B., AND R. K. MURTON. 1968. Photoperiodic and physiological adaptations regulating avian breeding cycles and their ecological significance. J. Zool. (Lond.) 155:327–394.

LOFTS, B., AND R. K. MURTON. 1973. Reproduction in birds, p. 1–107. *In* D. S. Farner and J. R. King [eds.], Avian biology. Vol. III. Academic Press, New York.

LUINE, V., F. NOTTEBOHM, C. HARDING, AND B. S. MCEWEN. 1980. Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. Brain Res. 192: 89–107.

MARSHALL, A. J. 1970. Environmental factors other than light involved in the control of sexual cycles in birds and mammals, p. 53–64. *In* J. Benoit and I. Assenmacher [eds.], La photorégulation de la reproduction chéz les oiseaux et les mammifères. Cent. Natl. Rech. Sci., Paris.

MARSHALL, A. K., AND H. J. DE S. DISNEY. 1957. Experimental induction of the breeding season in a xerophilous bird. Nature (Lond.) 180:647–649.

MARSHALL, A. J., AND D. L. SERVENTY. 1958. The internal rhythm of reproduction in xerophilous birds under conditions of illumination and darkness. J. Exp. Biol. 35:666–671.

MOORE, M. C. 1983. Effect of female sexual displays on the endocrine physiology and behavior of male White-crowned Sparrows, *Zonotrichia leucophrys*. J. Zool. (Lond.) 199:137–148.

MOREAU, R. E. 1950. The breeding seasons of African birds—1. Land birds. Ibis 92:223–267.

NEGUS, N. C., AND P. J. BERGER. 1977. Experimental triggering of reproduction in a natural population of *Microtus montanus*. Science 196:1230–1233.

NICHOLLS, T. J., AND G. P. GRAHAM. 1972. Observations on the ultrastructure and differentiation of Leydig cells in the testis of the Japanese Quail (*Coturnix coturnix japonica*). Biol. Reprod. 6:179–192.

OKSCHE, A., D. S. FARNER, D. L. SERVENTY, F. WOLFF, AND C. A. NICHOLLS. 1963. The hypothalamo-hypophysial neurosecretory system of the Zebra Finch, *Taeniopygia castanotis*. Z. Zellforsch. Mikrosk. Anat. 58:846–914.

PERRINS, C. M. 1970. The timing of birds' breeding seasons. Ibis 112:242–255.

PRIEDKALNS, J., AND R. K. BENNETT. 1978. Environmental factors regulating gonadal growth in the Zebra Finch, *Taeniopygia guttata castanotis*. Gen. Comp. Endocrinol. 34:80.

PRÖVE, E. 1974. Der Einfluss von Kastration und Testosteronsubstitution auf das Sexualverhalten männlicher Zebrafinken (*Taeniopygia guttata castanotis* Gould). J. Ornithol. 115:338–347.

PRÖVE, E. 1978. Quantitative Untersuchungen zu Wechselbeziehungen zwischen Balzaktivität und Testosterontitern bei männlichen Zebrafinken (*Taeniopygia guttata castanotis* Gould). Z. Tierpsychol. 48:47–67.

PRÖVE, E., AND K. IMMELMANN. 1982. Behavioral and hormonal responses of male Zebra Finches to antiandrogens. Horm. Behav. 16:121–131.

SANDERS, E. H., P. D. GARNER, P. J. BERGER, AND N. C. NEGUS. 1981. 6-Methoxybenzoxazolinone: a plant derivative that stimulates reproduction in *Microtus montanus*. Science (Wash. DC). 214:67–69.

SERVENTY, D. L., AND A. J. MARSHALL. 1957. Breeding periodicity in Western Australian birds: with an account of unseasonal nestings in 1953 and 1955. Emu 57:99–126.

SERVENTY, D. L. 1971. Biology of desert birds, p. 287–339. *In* D. S. Farner and J. R. King [eds.], Avian biology. Vol. I. Academic Press, New York.

SKADHAUGE, E., AND S. D. BRADSHAW. 1974. Saline drinking and cloacal excretion of salt and water in the Zebra Finch. Am. J. Physiol. 227:1263–1267.

SOSSINKA, R. 1970. Domestikationserscheinungen beim Zebrafinken *Taeniopygia guttata castanotis* (Gould). Zool. Jahrb. Abt. Syst. Bd. 97:455–521.

SOSSINKA, R. 1972. Besonderheiten in der sexuellen Entwicklung des Zebrafinken *Taeniopygia guttata castanotis* (Gould). J. Ornithol. 113:29–36.

SOSSINKA, R. 1974. Der Einfluss von Durstperioden auf die Schilddrüsen- and Gonaden-aktivität und ihre Bedeutung für die Brutperiodik des Zebrafinken (*Taeniopygia castanotis* Gould). J. Ornithol. 115:128–141.

SOSSINKA, R. 1975. Quantitative Untersuchungen zur sexuellen Reifung des Zebrafinken, *Taeniopygia castanotis* Gould. Verh. Dtsch. Zool. Ges. 67:344–347.

SOSSINKA, R. 1980. Ovarian development in an opportunistic breeder, the Zebra Finch *Poephila guttata castanotis*. J. Exp. Zool. 211:225–230.

SOSSINKA, R., E. PRÖVE, AND K. IMMELMANN. 1980. Hormonal mechanisms in avian behavior, p. 533–547. *In* A. Epple and M. H. Stetson [eds.], Avian endocrinology. Academic Press, New York.

STOREY, C. R., AND T. J. NICHOLLS. 1982. Low environmental temperature delays photoperiodic induction of avian testicular maturation and the onset of post-nuptial photorefractoriness. Ibis 124:172–174.

WINGFIELD, J. C. 1980. Fine temporal adjustment of reproductive function, p. 367–389. *In* A. Epple and M. H. Stetson [eds.], Avian endocrinology. Academic Press, New York.

WINGFIELD, J. C. 1983. Environmental and endocrine control of avian reproduction: an ecological approach, p. 265–288. *In* S. Mikami, K. Homma, and M. Wada [eds.], Avian endocrinology: environmental and ecological perspectives. Springer-Verlag, New York.

WINGFIELD, J. C., AND D. S. FARNER. 1978. The endocrinology of a natural breeding population of the White-crowned Sparrow (*Zonotrichia leucophrys pugetensis*). Physiol. Zool. 51:188–205.

WINGFIELD, J. C., AND D. S. FARNER. 1980. Control of seasonal reproduction in temperate-zone birds. Prog. Reprod. Biol. 5:62–101.

ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.

Department of Anatomy and Histology, University of Adelaide, South Australia. Present address of first author: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721. Received 10 February 1984. Final acceptance 7 September 1984.