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Final Report - A.F.A. Sponsored Research Endocrine Fecology of Immature Birds

EDITOR'S NOTE: The A.F.A. has a research committee that has been quietly at work for several years now. At present Dr. Susan Clubb is chairing the committee and under her direction much progress is being made.

All money donated to the A.F.A. for research is spent on research alone - none is diverted for any other purpose. The research committee evaluates various research projects and determines which are most appropriate for A.F.A. funding. There are now five research projects that are sponsored by the A.F.A. and that should ultimately provide data that will prove of great value to aviculture.

In 1982 Dr. Arden Berkowitz was awarded a grant to study the seasonal variation of sex steroids in young psittacines. The following paper includes some of Dr. Berkowitz's results. This paper is the first in a series of papers representing the results of various A.F.A. sponsored research.

by

Arden Bryan Bercovitz, Ph.D.,
Fred Frye, Jr. and Jim Bain
Research Department
Zoological Society of San Diego

Submitted for publication in
Watchbird, 10/14/83

INTRODUCTION

My dictionary defines ology, informally: "Any of various studies or concepts designed by terms ending in -logy and generically related." Examples are numerous: 1) physiology, study of functional living processes; 2) endocrinology, study of the physiology of endocrine glands; 3) fecalogy, study of the process of bowel and kidney wastes. Please, read that last example one more time. The term fecalogy™ [1] is a descriptive one and possibly unknown to you. I just invented it to describe my work and the study of birds through their droppings. The view from behind many rare, beautiful, imported birds has been amazing. The excrement that is useless to the bird may become more important than we ever knew it to be. Just think for a minute what can be learned about you by studying your garbage and waste materials. Modern archaeologists

have found that Garbology is quite revealing of human behavior. The story behind the study of excretory hormones in captive birds is no less amazing.

Bird droppings convey several clues about many common questions asked by exotic bird breeders. Two questions frequently asked of me include: 1) what sex is it? 2) when will it successfully reproduce? Before giving you the latest details I want to acknowledge your partial financial support of this work. The AFA Special Research Committee deserves credit for recognizing and financing valuable research.

Experimental studies with two of the world's most endangered species has produced some exciting preliminary results: 1) indicating a better way to safely identify the sex in California condors (*Gymnogyps californianus*) soon after they hatch; 2) providing a nondisruptive method for evaluating the reproductive condition of Puerto Rican Amazon parrots (*Amazona vittata*) as they became sexually mature.

FECAL STEROID ANALYSIS

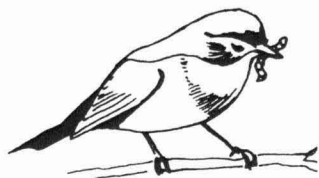
The analytic procedure for detecting changes in concentration of sex hormones in feces and urine of birds has been described for adult animals and successfully used to identify the sex in a wide variety of species (Czekala and Lasley, 1978; Bercovitz, et al., 1978; Stavy et al., 1979). The theory behind fecal steroid sexing is easily explained. The collected excrement is liquified, purified, hormone-analyzed and measured for total estrogens (E) and for testosterone (T), a major androgen. The final evaluation, as a ratio of E/T, can be used to identify adult males by their low E/T ratios, due to higher production and/or excretion of T by active testicles. Females can be identified by high E/T ratios, due to higher production and/or excretion of E by an active ovary.

In our last review, "Bird Sexing Methods - which should you choose?", (Bercovitz, 1981) I mentioned that the fecal steroid technique might not be capable of distinguishing either sex from birds that are immature, sexually inactive or birds with low total amounts of hormone due to age changes or for any reason. Age-related changes in steroid production from domesticated chickens provided evidence that active gonads were functionally distinct, male from female: 1) during the

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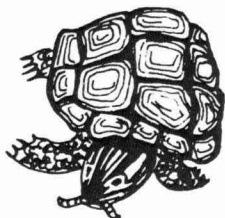
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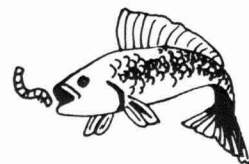


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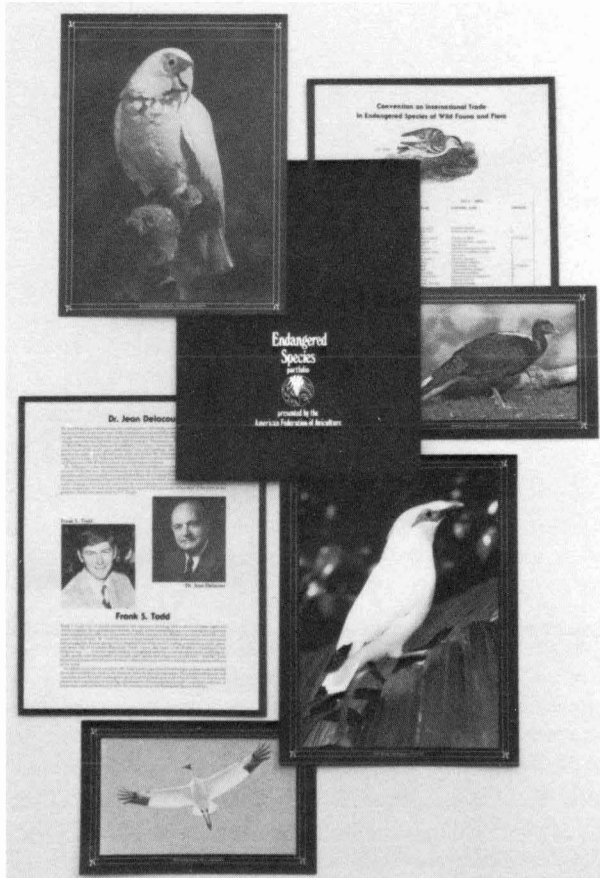
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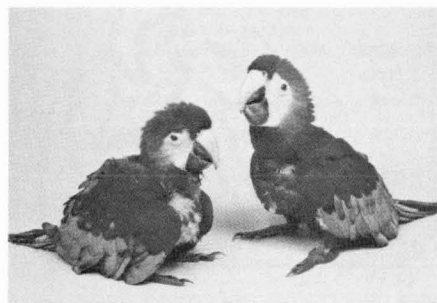
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last half of incubation; 2) during maturation of the reproductive process; 3) during annual breeding seasons. The total amount of steroid hormone and the specific hormone metabolites found in the droppings can provide important clues about what is going on inside the bird.

CALIFORNIA CONDOR SEXING

The past year has been one of monumental success with endangered species at the San Diego Zoo, i.e., the first artificially incubated and captive hatching of four California condors (*Gymnogyps californianus*). Photo A shows the first two chicks, photographed April 26, 1983; Sisquoc on the left hatched March 30 and Tecuya on the right hatched April 5. They are doing just fine, as you can see in Photo B. The same two birds, three months later, were growing at the tremendous rate of 8-10% 'daily' weight gains. I am pleased to recount some of the highlights of research with these unique birds, and development of a sexing technique for newly hatched condors.

Prior to hatching, there was much concern about the possibility of an abnormal sex ratio for the incubating condor eggs. Three California condors, trapped from the wild, were blood sampled and genetically sexed by chromosome analysis; all were males! Since the world population for this species is approximately two dozen birds, it

was essential to the success of our captive breeding program that at least one of the four eggs was a female condor. The rush to know their sex was arbitrary; they are not expected to reach maturity for another six to ten years.

Genetic sexing by chromosome analysis was scheduled and blood samples would be taken from the chicks when they were two months old and physically stronger than at hatching. Another project was planned to test if endocrine analysis of the excrement could be useful from materials found among the eggshell fragments and membrane residues. This kind of sample could be easily collected from the incubator without bothering the chicks. Concern for safety was a paramount issue, for nothing would be done to unnecessarily jeopardize these condor chicks. How soon after hatching could they be sexed? No one really knew and many things were considered including their coloration differences, rate of growth and behavioral responses. None of these data could definitely sex the four chicks.

Figure 1 illustrates the results from fecal analysis of the egg wastes from an Andean condor chick and the first three California condor chicks. The end result in this case was not a ratio of E/T, but a comparison of one particular type of estrogen, called estradiol. This hormone was isolated from other E's by a separation process called

Figure 1

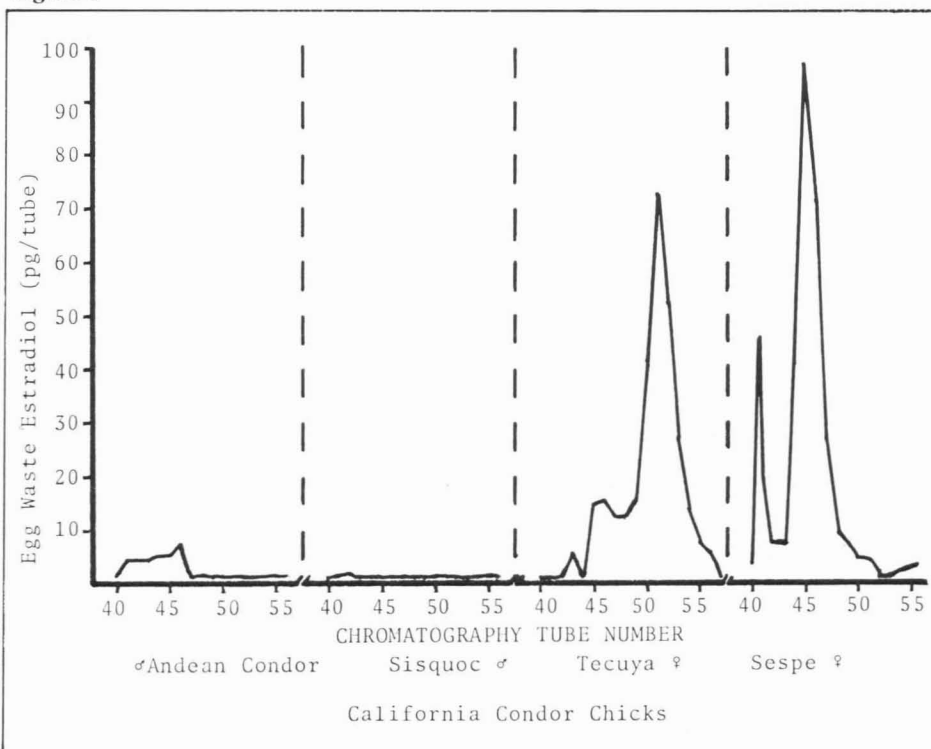


FIGURE 1. Egg waste estradiol analysis from two condor species. Fecal material collected at the time of hatching was purified and chromatographically separated to reveal only one component excretory estrogen, estradiol. Female condors presented eight to ten times higher values than did a male.



(A). The first two California condors ever artificially incubated and hatched in captivity. Sisquoc, a male on the left, was hatched March 30, 1983. Tecuya, a female on the right, was hatched April 5, 1983. This photograph was taken April 26.



(B). Sisquoc, again to the left, and Tecuya photographed August 1, have grown tremendously. Their average daily weight gains were 8-10%.

high performance liquid chromatography (HPLC). The difference between males and females, from egg waste estradiol, was obvious, females were eight to ten times higher than males. Similar data had been reported from the blood or gonads of domesticated chickens, as evidence that the ovary tissue was producing elevated estradiol levels in female embryos. Although, no one had ever looked 'down

stream' in chickens, we were the first to investigate the feces of endangered birds of prey. Birds of a feather may flock together, but they are not all alike. Proof in chickens is far removed from confirmation in condors. The ultimate sexing of these four condor chicks, one male and three females, was defined by chromosome analysis and confirmed, in the first three, by egg waste estradiol measurements.

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Figure 2

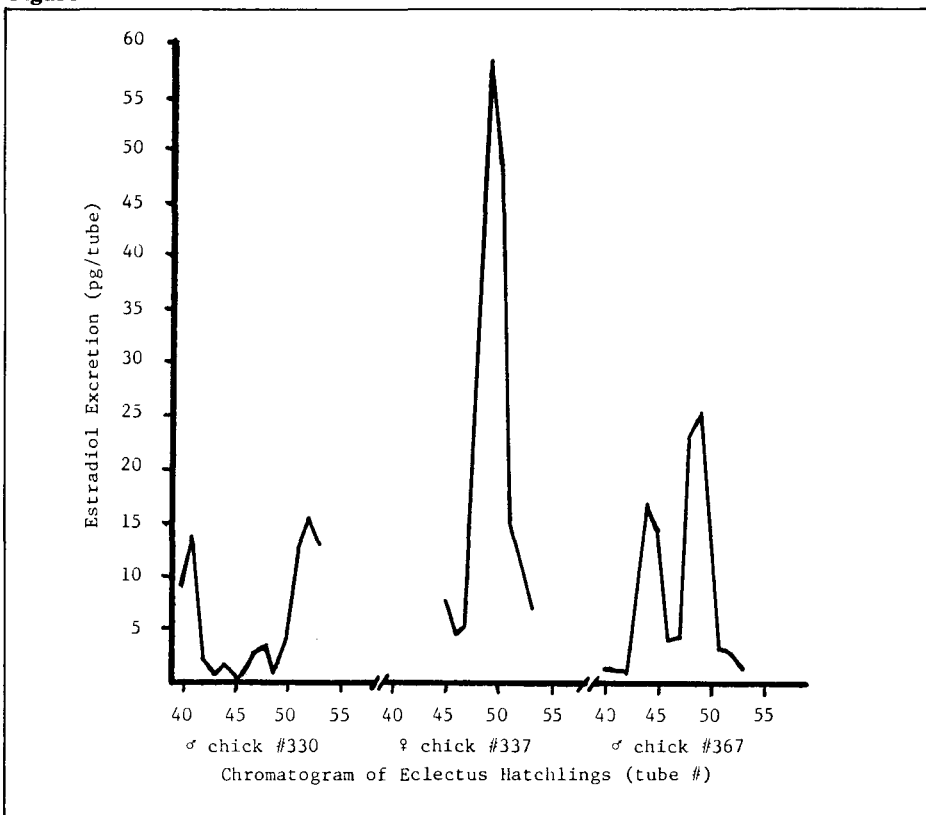


FIGURE 2. Excretory estradiol from Grand Eclectus chicks were collected and measured during the first week, post-hatch. The female presented two to four times higher estradiol levels than did males from chromatographically separated samples.

EXCRETORY ESTRADIOL FROM HATCHED PARROTS

Would the same thing work in parrot chicks? If bird embryos have distinctive gonadal functions, then what about the chick after hatching? Can they also be sexed by this method? Arrangements were made to collect feces from three Grand Eclectus parrots (*Eclectus roratus roratus*) during the first week post-hatch. That was not as easy as cleaning out the incubator for egg waste material. The chicks had to be handled several times a day and thanks to the diligence of Dr. Keven Flammer and the crew of Aviculture Institute, Newhall, CA. Samples were collected and sent to the San Diego Zoo for analysis. No need to guess about their sex; Photo C illustrates the obvious sexual dimorphism in this species.

Figure 2 illustrates the results of excretory estradiol measurements from two males and one female Grand Eclectus chicks. The sex comparison was not as clear cut as the condor egg waste estradiol data. The female Grand Eclectus had only two to four times more excretory estradiol than did the males. Closer evaluation of the excretory rate of hormones from these chicks indicated that by the end of their fifth day very little hormone was measurable in the droppings. The time frame for

using this procedure of sexing newly hatched chicks is apparently a short one. Much more work needs to be done to verify and simplify this method. That work is in progress and I will report the final outcome when the proof is available. These two pilot experiments were very encouraging. Is anyone interested in sexing their captive hatched parrot chicks?

MONITORING REPRODUCTIVE CONDITION OF PUERTO RICAN AMAZONS

At present the anticipated age at first breeding for 'parrots' ranges from two to seven years. Frankly, we just do not have a routine functional method to tell when any exotic bird is going to begin reproductive activity. Traditional endocrine techniques, involve blood or tissue collection have not been very practical, because of stress imposed upon the birds during handling and restraint. Fecal steroid analysis has been applied to the rarest of species, with no fatalities or serious injuries.

Too many parrots have the dubious distinction of endangered species status. The San Diego Zoo has been actively involved in a long standing cooperative project to save one species in particular, the Puerto Rican parrot (*Amazona vittata*). Acknowledgement and appreciation is deserved by Dr. James Wiley and Beth



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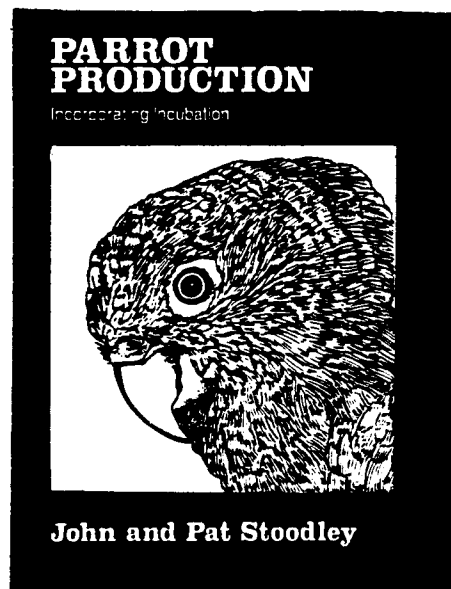
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Wiley for their assistance with sample collections throughout the years. You may remember the excellent report of this work (Wiley and Gee, 1981).

Fecal steroid analysis had been 100% successful in sexing adult Puerto Rican parrots. However, the data from juvenile birds is subject to further interpretation. Results from three year's collection were illustrated in Figure 3. Only two birds were sampled. They represented 10% of the world captive population. Equal concern for their safety was a crucial factor for choosing the noninvasive nature of repeated fecal collections.

Little evidence has been available about the onset of sexual maturity in Amazon species and our preliminary results (Figure 3.) require supportive work to fully evaluate. Here are the facts as we know them now: 1) females do mature earlier than males; 2) pair bonding occurs earlier than we might expect; 3) subadult birds display an increasingly distinct and more regular pattern in their excretory E/T values as they approached sexual maturity. The sex of these two birds has since been confirmed by an undisputed method, the production of eggs and a chick in 1982. The annual changes observed from E/T ratios from these two juvenile birds were more informative than any previous reports. Additional work in this area is necessary and in progress.

Photo courtesy of the Zoological Society of San Diego



(C). Adult Grand Eclectus parrots were once thought to be two different species, because of their striking sexual dimorphism. The green male is distinguished from the red female at hatching.

Figure 3

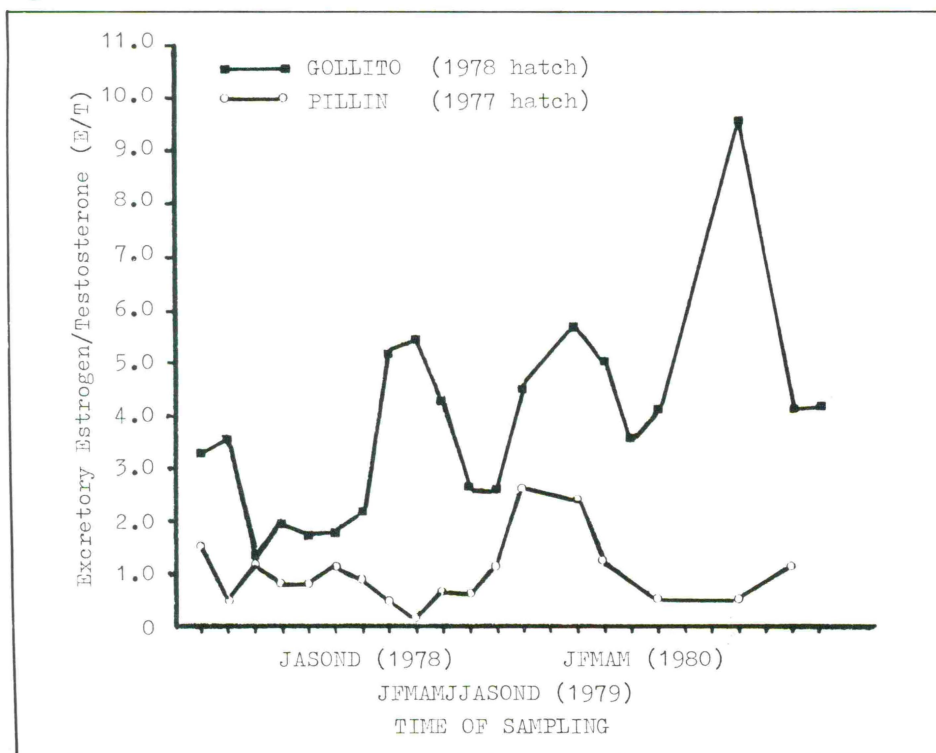


FIGURE 3. Annual, monthly changes in excretory ratio of total estrogen (E) and testosterone (T). Note the distinction E/T values between these two juvenile birds. Pillin, the male, was a year older than Gollito. This pair successfully produced eggs and a chick during the subsequent year of 1982.

## SUMMARY

I hope that your curiosity has been peaked and that I have justified your support of these preliminary findings. There is much more to learn about these fascinating creatures and some of the answers may be figuratively at our feet, especially that stuff we regularly just throw away. The sex in newly hatched chicks can be determined and information about the reproductive condition of immature birds can be obtained without harm or disruption, as we have demonstrated with two of the most endangered of all species. Future refinements may provide the means of developing this technology so that it can be broadly utilized. The prospects look very good.

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