Health and Reproductive Assessment
OF SELECTED PUERTO RICAN PARROTS *(AMAZONA VITTATA)*

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Figure 1. Puerto Rican Parrots *(Amazona vittata)* in the Iguaca Aviary. This large flight cage is used for conditioning, predator avoidance training, and food recognition training of young birds in preparation for release. Photo by Pablo Torres-Baez, staff-US Fish and Wildlife Service.

Abstract

Captive production of the Puerto Rican Parrot *(Amazona vittata)* has been instrumental in recovery of the species from only 13 individuals in the wild in 1975 to a total population of approximately 500 in 2015, (20 to 25 in the wild population in El Yunque National Forest, approximately 100 in the wild population in Rio Abajo State Forest, and over 415 in the captive breeding facilities of the Luquillo/Iguaca and Rio Abajo aviaries). The Puerto Rican Parrot has become an iconic and high-profile conservation species. The cornerstone of the recovery plan for this critically endangered species is an active captive breeding program, management of the wild population, and long-term reintroduction program. The two aviary populations in Puerto Rico (Iguaca, formerly known as Luquillo, and Río Abajo) were this health and reproductive assessment of a sub-set of the species took place, are the only source of parrots for release.

Reproductive assessment is a tool used to enhance reproductive potential and produce productive pairings. In 2002 a number of adult birds, which had not produced viable offspring were selected for reproductive assessment, in an attempt to augment the population growth and provide ample individuals for re-introduction. Normal productive individuals were not examined. This assessment included thorough physical exam, endoscopic evaluation, hematology and blood chemistry profiling, viral screening and hormonal assessments.

Key words: *Amazona vittata,* Puerto Rican Parrot, psittacine reproduction, endoscopy.

Introduction

The dramatic reduction in the population of the Puerto Rican Amazon (PRP from here on) during the 19th century led to its designation as a critically endangered species in 1967[1,2,3,4]. While the primary cause for this decline is habitat destruction[1], low rates of reproduction in the wild as well as captive flocks of Puerto Rican parrots have contributed to the decline of the species[2,3,4]. As part of a coordinated conservation program for the species, the U.S. Fish and Wildlife Service and the Department of Natural and Environmental Resources cooperatively manage two captive breeding centers for the species in Puerto Rico[2,3,4]. Figure 1. This study was designed to investigate causes and solutions for low reproductive performance in captive pairs of birds.

Avian infertility can be associated with numerous factors and possibly a combination of factors. These factors may be behavioral, environmental or medical[5,6]. Behavioral causes of infertility in captive parrots can include, pair incompatibility, sexual inexperience, lack of early learning, aviary disturbances, lack of social interaction, excess social interaction, homosexual pairs, lack of pair bonding, asynchronous breeding condition, improper imprinting, and infrequent matings[5,6].

Environmental causes can include incorrect photoperiod, incorrect nest box or nesting materials, incorrect enclosure design, lack of visual barriers, excessive rain, temperature or humidity, storms or other disruptive climatic events, disturbance by pests or predators, lack of appropriate foods, and loose or incorrect perches[5,7].

Medical or physical causes of infertility can include physical disabilities, obesity, age (too young or old), inbreeding, chronic hypovitaminosis E, drug therapy (causing vitamin deficiency or direct decreased fertility), hormonal abnormalities, musculoskeletal, neuromuscular or other disease (causing pain, paresis, ataxia, weakness, decreased muscle control), reproductive tract disease, nutritional deficiencies or excesses, systemic diseases, parasitic disease leading to malnutrition, cloacal abnormalities including excessive feathering, possible thyroid deficiency, and toxins (mycotoxins, pesticides, chemicals)[5,6,7,8,10].

Materials and Methods

In November 2002 this study was initiated in an attempt to define causes of low reproductive performance in selected Puerto Rican parrots, specifically low male fertility. Examinations were conducted in the Luquillo (now Iguaca Aviaries) and Rio Abajo Aviaries. A portion of the captive population, 47 birds (26:21) were examined systematically with data recorded and analyzed using Excel software. Thirty-eight birds were housed at Luquillo Aviary (21:17) and 9 at Rio Abajo Aviary (5:4). The birds ranged in age from 1 to 34 years (Ave 13 y). Seven (4:3) were Hispanolian Amazons *(Amazona ventralis)* that were used as surrogate parents for Puerto Rican Parrots.

Birds were anesthetized with isoflurane for the physical exam and endoscopy. They were placed in right lateral recumbence with the left leg extended, and the left flank area was prepared as a surgical field. The body wall was penetrated just caudal to the last rib on the left using a sharp trochar in a cannula, and a rigid 2.2 mm Storz® arthroscope was inserted for a visual examination[10]. No closure was required as the tissue plains shifted and sealed the puncture hole when the leg returned to a normal position.

All birds were examined for oral and cloacal papillomas. Plumage condition was evaluated by visual exam of feathers for completeness, color, and condition of the plumage. Blood samples were collected for hematology using EDTA as anticoagulant, plasma biochemistry using lithium heparin as an anticoagulant, disease screening, resting thyroxine (T4) and testosterone levels in (males)[6,8,9].

Ovaries were assessed visually by endoscopy and scored (1-4) in the following categories: stroma (mass of small follicles) size, follicle number, follicle size, amount of retained yolk material indicating the presence of post ovulatory or atretic follicles, oviduct hypertrophy indicating preparation for egg production, density of membranous covering of ovary and flaccidity of the vent also indicating preparation for egg laying.

Testicular morphology was subjectively evaluated using a scale of 1-4 for the following parameters: relative size (4 is the largest), vascularization/ hyperemia- with 1 being normal white color to 4 being reddish or hyperemic in color, texture of the surface on scale of 1-4 with 1 being normal, smooth serosal surface to 4 being irregular with numerous wart-like serosal and/or parenchymal irregularities, testicular density or opacity, and visualization of vas deferens. Adrenal size relative to testicular size was expressed in a ratio, with 1:1 being the testes were the same size as the adrenal gland. This ratio may indicate that the testes were small or the adrenal gland was enlarged, or could be normal in an immature bird.

All birds were tested for *Mycobacterium* (group specific antigen) *Chlamydophila* (group specific antigen), avian *Polyoma* virus, psittacine *Circovirus,* psittacine *Herpes* virus associated with Pacheco's Parrot Disease, and *Salmonella* (group specific antigen). Avian Biotech Laboratories, Tallahassee, FL, performed disease screening by PCR analysis of DNA extracted from whole blood. All birds were found to be negative on all assays. All blood samples were negative for blood parasites by standard microscopic exam of blood smears performed by the Avian & Wildlife Laboratory, University of Miami, FL. Biochemical analysis was performed by Abaxis Vetscan® analyzer using whole blood. The International Species Information System (ISIS) supplied normal values used for Amazons. Aspartate aminotransferase (AST) was not performed in 23 birds because of sample hemolysis. Resting plasma thyroxine (T4) and testosterone levels were determined for each bird by radioimmunoassay by the Bronson Diagnostic Laboratory, Kissimmee, FL.

Paired skin/feather biopsies were performed in 7 birds with obvious feather abnormalities associated with feather plucking. Paired biopsies allow a comparison between affected (plucked area) and unaffected areas[11]. Skin biopsies were fixed in formalin, processed routinely, sectioned at 3 levels at 5µm thickness, and stained with hematoxalin and eosin[11].

Results

General condition was judged by physical examination to be excellent in 31 birds (65%), good in four birds (8.5%), fair in 8 birds (17 %) and poor in 3 birds (6.3%). Assessment of body condition by keel score was ideal (3 on scale of 1-5) for 39 birds. Only 2 birds were considered slightly overweight and 5 were slightly underweight. Twenty nine birds (61%) had excellent plumage condition, 8 (17.0%) were good to fair (21.2%) and 10 showed feather damaging behavior, 2 of which were plucked on the head by their mates. The mean body weight for all birds was 297 grams (range 239- 398), in females was 292 grams (range 239-399), and in males was 302 grams (range 245-398).

No evidence of internal papillomatous disease was seen. One bird had previously been diagnosed and treated for cloacal papillomas but no evidence of papillomas was observed on this exam. Two years later the bird died and no evidence of papillomas was found on necropsy.

Upon endoscopic exam the air sacs of 38 birds were clear and normal. One bird had cloudy air sacs, one had lesions suggestive of previous aspergillosis, and in one bird the air sacs were very vascular with floating debris. This bird was isolated; the air sacs cultured and were subsequently treated for a *Pseudomonas aeruginosa* infection. Examination of the left cranial thoracic, caudal thoracic and abdominal air sacs, and organs were normal in all birds with the exception of white foci in kidneys (1 bird), enlarged spleen (1 bird) and mild anthracosis in the lungs (3 birds).

The proventriculus appeared enlarged in 22 (10.12) birds (46%), but in several birds it appeared to become reactive to the touch

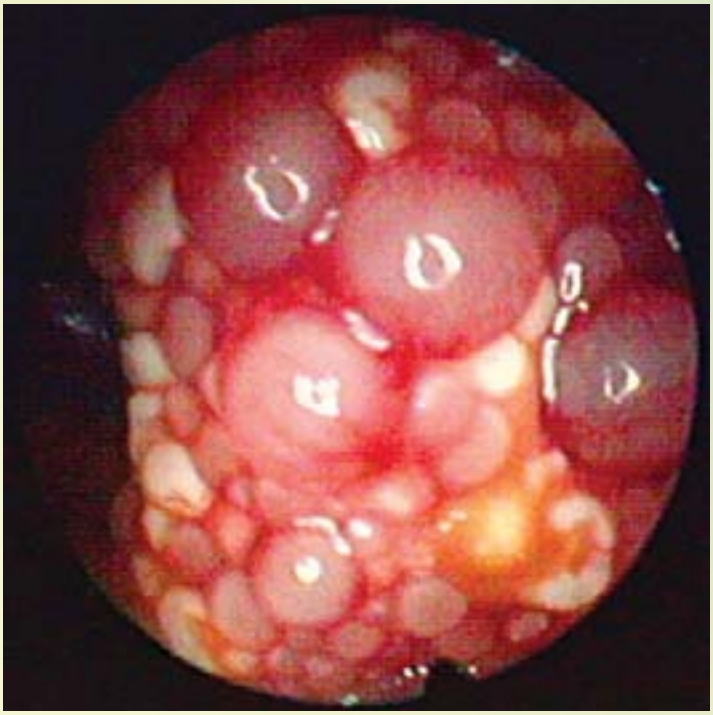

Figure 2. Small and medium sized follicles are seen in this ovary of a sexually active female. Yellow material probably represents post ovulatory follicles. This female would have excellent reproductive potential.

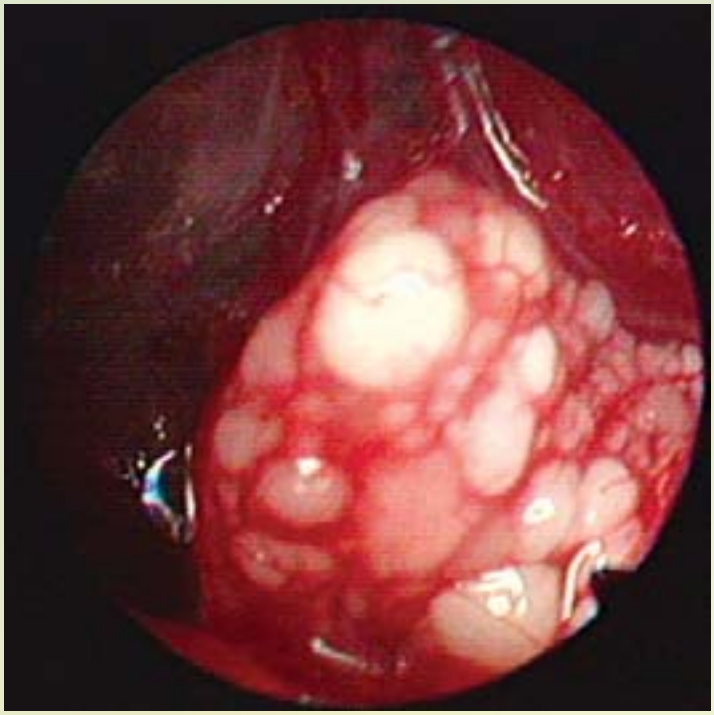

Figure 3. In this 29-year-old hen, retained material appeared white rather than the normal creamy or yellowish material. It was biopsied and identified as fibrotic tissue. This bird probably has little potential for further egg production and could be considered senescent.

of the endoscope and visibly became more distended during the examination. The birds were fasted prior to examination so this enlargement was unlikely to be due to engorgement with food. The cause for this proventricular enlargement is unknown. This observation of proventricular enlargement is a very subjective observation and may be an idiosyncrasy of this species. One explanation for proventricular enlargement may be the dose dependent effect of isoflurane on smooth and skeletal muscle relaxation. Proventricular enlargement did not seem to correlate with any specific age group (ages 1-28 years, mean 13 years). General body and feather condition of birds with proventricular enlargement did not seem to vary from that of the total population. The gonadal condition did roughly correlate with proventricular enlargement. The ovaries of females with proventricular enlargement appeared to be more active however this is a very subjective assessment. Histopathology is performed on every bird that dies at these aviaries and proventricular dilatation disease (PDD) has never been found in this population.

Four birds had variable yellow foci observed in the liver parenchyma (multifocal). At the time of the examination these spots were thought to indicate possible mycobacterium infection (tuberculosis) however all birds tested negative by PCR analysis of whole blood. Livers were not biopsied due to malfunction of biopsy forceps. Sexes of birds with yellow foci in liver were 1.3, and ages were 4-17 yrs. Mean body weight for the 4 birds was 310 gm (well above average for population). Three females had very active follicular development indicating that these foci may be fatty in nature and possibly associated with mobilization of reserves in association with yolk formation. Hematology and plasma biochemistry values did not suggest infectious disease.

The ovarian stroma and its mass of varing sized follicles was generally normal in most of the females. Figure 2. It was small in 4 birds (1, 14, and 2 birds 29 years old). The small stroma in an older bird may be attributed to depletion of embryonic follicular sites and exhaustion of the potential for ovulation.

The number of visible ovarian follicles was estimated subjectively on a scale of 1-4. Two birds were scored 1+ (both age 29) and had very few visible ovarian follicles, 5 birds were scored 2+ (age 3-26, mean 14 years), 6 birds were scored 3+ (age 1-14, mean 9 years), and 7 birds were scored 4+ with large numbers of follicles spread evenly across the stroma (age 4-23, mean 12 years). In this assessment the 2 older birds again appeared to have at least partially exhausted their reproductive potential; however the breeding age birds showed good potential for active egg production.

Ovarian follicle size was subjectively assessed again on a scale from 1 to 4. Four birds were scored 1+, no enlarged follicles (age 3-29, mean 19 years), 6 birds were scored 2+ (age 1-19, mean 9 years), 7 birds were scored 3+ (age 4-23, mean 10 years), and 1 bird (age 8) was scored 4+ with many large follicles. Since the active period of egg laying for the Puerto Rican parrot is in the spring, this evaluation in November was indicative of excellent reproductive potential for females in the population. One bird had a moderate number of follicles containing dense and white material (age 29). Figure 3. This ovary was biopsied and found

Figure 4. Thickened membranes partially obscure this ovary, and may be an indication of an inflammatory process in the ovary.

to contain inactive proteinaceous material consistent with a senescent ovary. In this assessment the very old birds again appeared to have limited reproductive potential. Only 1 bird showed very large follicles, however this would not be unusual in the non-breeding season.

The presence of retained yolk material, presumed to be post ovulatory follicles or atretic follicles, was noted in 18 females and was again estimated on a scale of 1 to 4 with the additional notation of negative or none seen in 5 birds (age 1-29, mean 17 years), 2 birds had 1+ (age 7 and 14), very little retained yolk material, 8 birds had 2+ (age 7-26, mean 15 years), 2 birds had 3+ (age 8 and11), and 1 bird showed white scaring (age 29).

Most females had little oviduct hypertrophy, which would be expected in the non-breeding season. Involution of the oviduct is normal when the birds are not in an active phase of reproduction and can correspond to dramatic seasonal fluctuations in body weight.

Inflammatory disease may be associated with thickening and opacity of membranes surrounding the ovaries and may indicate advanced age, infection, or non-specific inflammation. Figure 4. Opacity or thickening of ovarian membranes was estimated on a scale of 1 to 4 with 1 being no visible membranes to 4 being very thick membranes obscuring visualization of the ovaries. Most birds appeared normal. Two birds had 2+ membranes (age 26 and 29), and one bird had 4+ membranes obscuring visualization (age 23), possibly associated with an undiagnosed inflammatory process.

Hypertrophy of the vent tissue, a common finding in sexually active females, may indicate hormonal conditions conducive to egg production and is a seasonal physiological change in reproductively active females. This change was noted in 10 females (ages 3-20, mean10 years). Surprisingly the incidence of birds showing hypertrophy and flaccidity of the vent seemed much more indicative of reproductive readiness than was represented by follicular enlargement observed by direct endoscopic examination of the ovaries.

Testes were evaluated and in 4 birds testicular biopsies were attempted. Unfortunately, the biopsy forceps were not functioning properly and biopsies were only useful in 2 birds. Testicular biopsy poses some risk of damage in testes that are active and hypertrophied[8,12]. Leakage of tissue could theoretically result in an autoimmune reaction against the bird's own testicular tissue[8,12]; therefore biopsies were only attempted in testes that appeared to be abnormal or atrophic. In the author's experience, testes that are atrophic do not leak post-biopsy[12].

Testicular size was subjectively estimated. The degree of seasonal variation which would be expected is not known because breeding birds are not typically evaluated during the breeding season as this disruption would likely result in subsequent loss of production. Despite the seasonal variation, the relative testicular size seemed to correlate with expected observations as associated with age (immature birds and advanced age). An unexpected finding was a 34-year-old male with very large active looking testes. Four birds had 1+, small testes (age 2-27, mean 9 years) 11 birds had 2+ size testes (age 4-18, mean 11years), 7 birds had 3+ sized testes (age 8-23, mean 15 years), and 2 birds had 4+ very large testes (age 18 and 34).

Vascularization and hyperemia of the testes was subjectively assessed. This is an important observation because orchitis is often evident by visual hyperemia of the testes and is a common finding in male Amazon parrots[5,6,7,8,12]. Twenty two males had normal colored testes that would indicate a relatively low level of orchitis. Only one bird had 4+ hyperemia.

The texture of the testicular surface/presence of cyst-like structures or irregularities was evaluated. These cyst like structures can be an indication of tubular abnormalities, dilations of tubules, which may or may not affect fertility[5,12]. Fifteen birds showed normal, smooth surface texture. Six birds had slight irregularities in texture or surface (age 3-16).

Testicular density or opacity is a subjective measure of the amount of fibrosis in the testes. Normal testes appear uniform and translucent[8]. Amazon parrots with orchitis tend to develop fibrosis of the testicular tissue as sequelae to chronic inflammation as indicated by opaque irregular appearance of the tissue[12]. Three birds, showed increased 3+ density of testes (age 5-27, average16 years). In one young bird the testes appeared very hard and dense (age 4). This is a very uncommon finding in such a young bird. The significance is unknown.

The appearance of the vas deferens was noted when visualized. Abnormalities of the vas deferens are occasionally observed endoscopically, including cystic structures or apparent obstructions. These abnormalities were not observed in any birds.

The adrenal: testicular (A:T) size ratio is a subjective visual comparison of size of the adrenal gland to testicular size. (Clubb, 2002) If A:T = 1:1, the testicle is the same size as the adrenal gland. This is considered normal in immature birds but in adults may be an indication of stress related suppression of testicular function or adrenal hyperplasia in response to chronic stressors. A:T ratio of 1:1 was found in 8 birds (age 2-28, mean12 years). If A:T = 1:2 the testicle was twice the size of the adrenal gland. This was found in 5 birds (age 4-16, mean 9.4 years). A:T = 1:3 was found in 4 birds (age 8-18, mean 14.5 years). A:T = 1:4 was found in 3 birds (age 8-23, mean 14.5 years). A:T = 1:5 or 1:6, indicating very large testes was found in 3 birds (age 11-34, mean 21 years).

The membrane density around the testes was subjectively assessed as a general indication of inflammation. Increased density and opacity of peritesticular membranes may be associated with inflammation such as orchitis. Most of the birds had no visual opacity or hypertrophy of peritesticular membranes. Only 1 bird had a 3+ score.

Thyroxine (T4) analysis was performed in 37 birds. The range of T4 values was 0.07 -1.8 µg/dl (mean was 0.50 with standard deviation of 0.40). The reference range for Amazons as established by University of Miami Department of Comparative Pathology is 0.1-1.1µg/dl. T4 values in females (13 birds) were 0.17-1.16 µg/dl (mean 0.47), and in males (24 birds) were 0.07-1.8 µg/dl (average 0.52). Two male birds (age 4 & 17) fell below this range. Four birds, 1 female (age 26) and 3 males (age 3, 16 and 27) had T4 values above the reference range. As the birds were evaluated at different times of the day, some variation might be expected to be associated with diurnal variation. No data is available to evaluate temporal variation.

Analysis of plasma testosterone levels in male birds revealed no testosterone activity in 15 of 20 samples. Of the 5 birds with detectible testosterone, all were below reported normals except one bird and that bird was 34 years old. Testosterone levels for the PRP were very low compared to reference ranges. Reported normals for 10 avian species almost without exception exceeded 0.2µg/ml[8]. Similar values, typically greater than 0.2 µg/ml, were

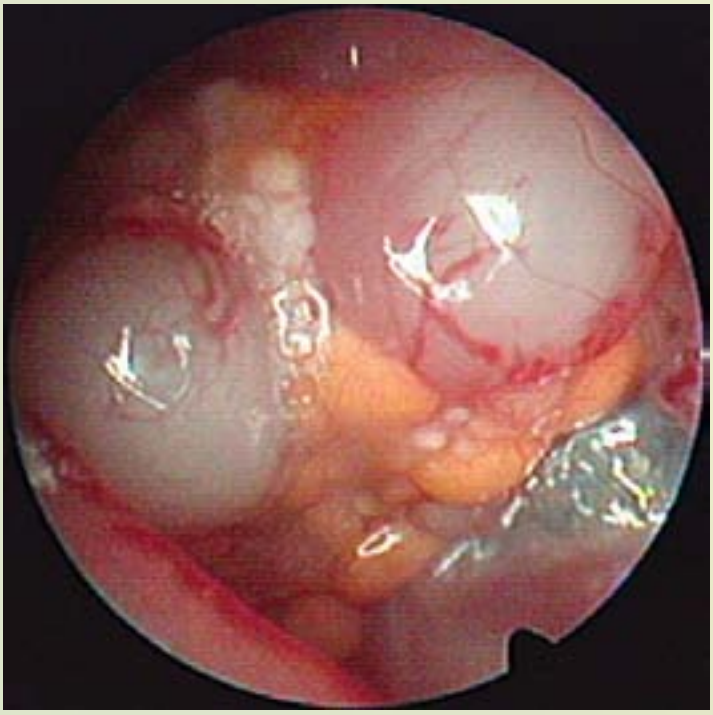

Figure 5. This very active ovary shows a mixture of large, medium and small sized follicles as well as post ovulatory follicles (yellow) and fibrotic follicles (white).

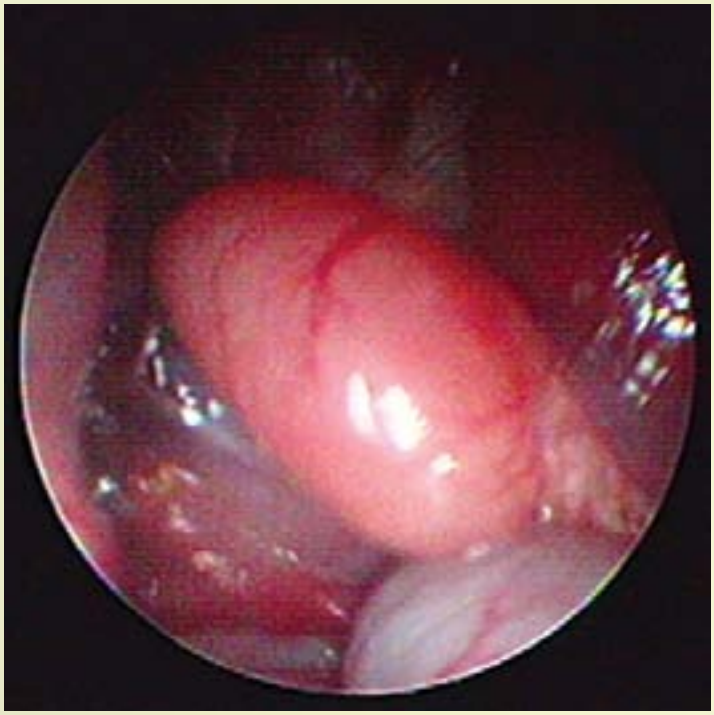

Figure 6. The large active testicle of this bird obscures the Adrenal gland.

reported for six avian species by Van Teinhoven[13]. Testosterone levels are noted to be very variable seasonally. Inter-lab and intra-lab variations are also well documented.

Plasma glucose levels, total protein, albumin, globulin, AST (in those individuals tested) and uric acid levels were within normal ranges. Creatine kinase (CK) levels were available for 32 birds and ranged from 183 to 4589 U/L. CK levels in 22 birds were clearly in excess of the reference ranges for amazons, which are 55-345.

Discussion

General physical examination, hematology and plasma biochemistries of this subset of the populations of birds, at both the Luquillo (now Iguaca since 2007) and Rio Abajo Aviaries, revealed overall good health and condition. No major infectious diseases were found. This would indicate that a high level of general husbandry, nutrition and management is being practiced at both aviaries. Endoscopic examination also revealed overall good health and condition, especially of females. The apparent low fertility of male birds warrants further investigation.

Most of the females showed good overall reproductive potential, consistent with maturity during the non-breeding season. Figure 5. Birds in their late 20's might be considered functionally senescent with low expectation of reproductive success; possibly indicating the reproductive life span may be limited to perhaps 30 years of age. Examination and review of breeding records of more females of this age would be needed to validate this observation. The longest known lifespan of a Puerto Rican Parrot is estimated to be 40 years based on a single wild caught male bird (Personal observation-Vélez).

Retained yolk in post ovulatory sites is an indication of previous ovulation[9]. This material often accumulates in older birds (Clubb-personal observation). The ovaries in 2 older birds (29 and 20) were biopsied and pathology revealed proteinaceous material, possibly granulomatous, with inflammation and fibrosis. In the 29 year old bird the retained material appeared very white rather than the normal creamy or yellowish material and was more consistent with fibrosis. In the 20 year old bird the material was more consistent with a post ovulatory follicle and mild inflammation and thickened membranes overlying the ovary. It was not determined if the accumulation of this material is consistent with a pathological change or rather is a normal physiological finding in multiparous females. Two additional ovarian biopsies revealed cystic appearing follicles and inflammation.

Ideally in reproductively active male parrots the testes become so hypertrophied that the adrenal gland is totally obscured from view[8], or if it can be visualized the A:T ratio will often range from 1:3 to 1:6. Figure 6. This can also be a subjective indication of stress. It is theorized that stress and the subsequent endogenous glucocorticoids can suppress seasonal testicular hypertrophy. Therefore in a visual comparison of the adrenal and testicular size, if the adrenal gland is hypertrophied and the testes are small this may be an indication of stress related suppression of testicular function. Figure 7. This is a **very** subjective analysis but as the testes cannot be directly measured

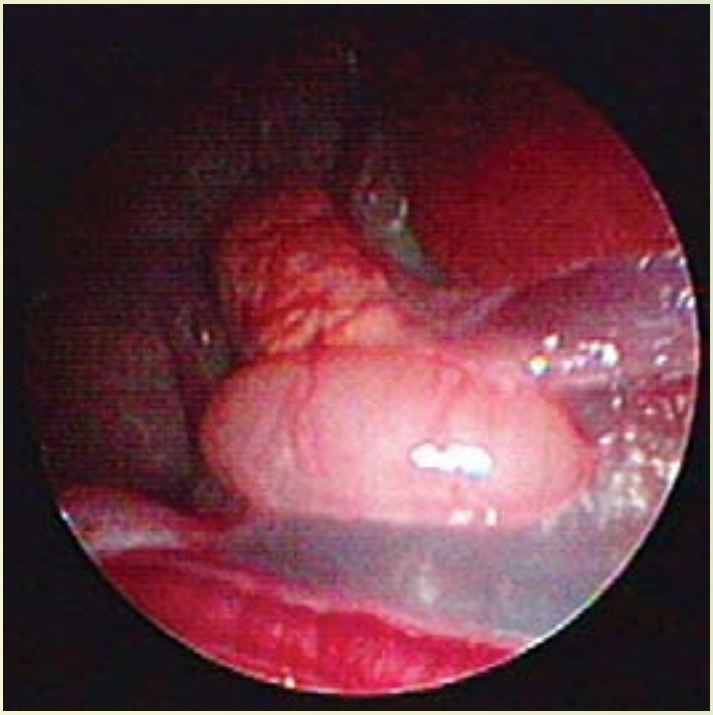

Figure 7. The enlarged adrenal gland in this male bird is roughly equivalent in size to the testicle. This would be an A:T ratio of 1:1 (adrenal/testicular size ratio) which could indicate a stress response and subsequent poor reproductive performance.

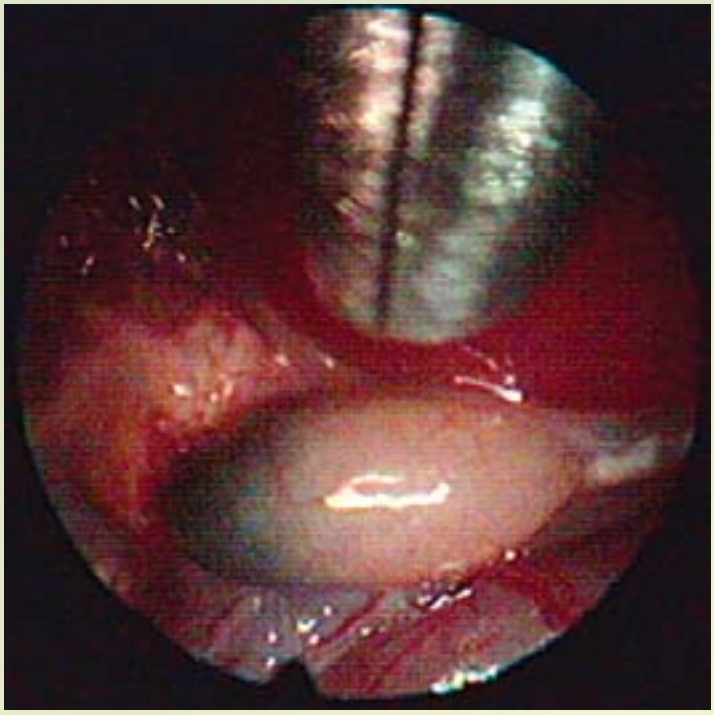

Figure 8. Biopsy forceps approach a small, dense testicle. This bird had fibrosis believed to be secondary to chronic orchitis. A:T ratio in this bird would be 1:1.

as in reproductive assessment in mammals, this visual judgment gives a basis for comparison of males. It would appear from this analysis that the greatest reproductive potential for males is during the teen years which is consistent with some other psittacine species[7]. The very active 34-year-old bird may be an indication of prolonged reproductive potential or might be an individual variation. Despite his apparent fertility, he has only produced one offspring.

Two testicular biopsy samples had nicely preserved testicular tubules, neither with mature sperm. Both showed mild capsular chronic inflammation and mild (< 5%) fibrosis. Figure 8.

Plasma testosterone has been reported to vary with age, seasonally, with changes in photoperiod, molting cycles, courtship behavior, feeding of young, and pair bonding[8]. The seasonality of plasma testosterone levels in parrots has not been well documented but in our case this preliminary finding appears to be significant and certainly warrants further investigation. These low testosterone levels could be a reflection of the non-breeding condition of the parrots in the fall. A logical next step in this investigation would be to sample plasma testosterone levels in males if they were captured for any reason during the breeding season. Special attention should be placed on birds showing aggressive behavior.

In all 7 birds that had paired skin/feather biopsies histopathologic examination revealed perivascular dermatitis and possible hypersensitivity which may have resulted in the feather plucking. These birds were 4 males (age 15-34, mean 25 years) and 3 females (age 14-20, mean 15 years). It would be interesting to examine the relatedness of these birds in an attempt to determine if hypersensitivity might be genetically predisposed in these individuals. Medical management for control of hypersensitivity might provide symptomatic relief and temporary improvement. Long term management may be required for return of plumage but would be dependent on chronicity and the degree of irreversible damage to feather follicles. There did not appear to be an age correlation with T4 level or with hypersensitivity dermatitis.

The apparent enlargement of the proventriculus and elevated plasma CK levels in many of the birds leads to a concern about the potential presence of proventricular dilatation disease (PDD) in the population. It is also possible that this is a normal variation in the species and does not indicate any underlying disease process. This is an area that warrants further investigation. The lack of previous cases of PDD in this

population as determined by necropsy records supports the assumption that these findings are not indicative of this disease being present in the population. The yellow foci in the liver, as detected endoscopically, are also of mild concern, despite the negative PCR for mycobacterium in all birds tested. Targeted hepatic biopsy would provide definitive information.

There does not appear to be an obvious correlation between enlarged proventriculus on endoscopy and elevated CK levels. They also appeared to be independent of sex or age. Reported reasons for elevation of plasma CK levels include blunt trauma, capture myopathy, exertion, feather picking, furazolidone cardiotoxicosis, irritating injections, nutritional myopathy, self-mutilation, starvation, or surgery[14]. Proventricular dilatation disease can also result in elevation of plasma CK levels[15]. Blood was collected after the endoscopic procedure however it would appear to be a relatively short time for tissue trauma associated with endoscopy to cause such a dramatic increase. A review of medical records since this evaluation demonstrated that elevation of CK is a very common non-specific finding in the species, possibly associated with capture and restraint.

Low male fertility was the impetus for this study and findings indicate some areas of concern. Orchitis does not appear to be causative in this population as indicated by these findings. However, low testosterone levels are of concern, even during the non-breeding season. This correlated with a relative high percentage of birds with small testes. This is a subject that certainly warrants further investigation. Thyroid dysfunction did not appear to be involved as T4 levels were generally within the normal range.

In a report entitled *"Sex and Age Structure of Puerto Rican Parrots at the Luquillo Aviary"* and a "Recommended Management Strategy- 1987-1991" by Dr. Jim Wiley[16], the theory of psychological castration of males was proposed. In the 1987 management strategy infertility was addressed and it was suggested, "males may be psychologically castrated by being maintained in adjacent cages without a sense of secure territorial boundaries between pairs"[16]. Visual barriers were installed in an attempt to correct the problem. Dr. Wiley also recommended pairing young males with older egg laying females. A successful Amazon breeder in Loxahatchee, FL (Voren, personal communication) feels that Amazon species with red frontal coloration and red wing speculums use these color patches in sexual displays both with females and other males. It is his opinion that housing breeding males that have red frontal coloration in close proximity to each other may suppress reproduction. He recommends keeping pairs of other Amazon species, without red frontal coloration in adjacent cages to reduce this phenomenon. This would appear to correlate well with Wiley's theory of psychological castration[16]. The suspicion of low male virility in the apparent absence of disease is supportive of behavioral or physiological factors being of great importance.

Theoretically pherhormonal suppression of subordinate males by a dominant male may ensure reproductive dominance. This may have occurred in this population. Certainly genetic factors must also be considered. Another finding that further supports this theory is that unpaired male PRP, maintained in bachelor groups, at both aviaries were often found to have larger more active testes than some of the males in pair breeding cages.

The different management style present at the Rio Abajo aviary, with pairs in relatively larger breeding cages spread out through forested trails has been rewarded by a greater reproductive success. This fact further supported the theory of psychological castration and its importance for the birds in the Luquillo Aviary as existed at the time of the study. Proposed changes included larger cages, visual barriers and vegetation between cages to reduce psychological or possibly pherhormonal reproductive suppression. The possibility that housing of pairs in close proximity may suppress the reproductive potential of males was considered and changes were implemented with positive results in the construction of the new Iguaca Aviary.

Flocking of breeding birds in the non-breeding season is another management tool that has been shown to enhance breeding success of Amazons. A similarly colored and also endangered species, the red-browed amazon *(A. rhodocorytha)* showed greatly enhanced breeding success in years subsequent to flocking in the non-breeding season, at Loro Parque, Tenerife, Spain (Clubb-Personal observation). Even if birds do not form new pairs, the social stimuli of natural seasonal flocking provides tremendous psychological and sexual stimuli that could result in enhanced reproductive performance. Flocking is now being practiced using juvenile PRP of the current season with other immature and mature birds. Seasonal enrichment of the diet with natural foods such as Sierra palm seeds *(Prestoea montana),* Cupey *(Clussia clusoides, Clussia rosea, Clussia grisebachiana),* Tabonuco seeds *(Dacroydes excelsa),* Palo de Cruz *(Redia portoricensis),* Guaraguao seeds *(Guarea guidonea),* and many others, as well as supplementation with additional Vitamin E provided further psychological and physiological enrichment for breeding males at the time they are moved into the new breeding enclosures.

Following this assessment changes were made in housing for breeding pairs at the Luquillo Aviary to enhance male reproductive performance. Endoscopic examination in this case proved to be a useful management tool to enhance breeding success of the PRP. As a result of this study, 3 non-producing pairs were re-paired and 5 new pairs were formed with positive results. Thirty-two fertile eggs and 22 hatchlings were produced between 2003 and 2005 as a direct result of these new pairings. This significantly increased the captive population of PRP at the Luquillo Aviary. A second reproductive assessment was performed in 2005.

The Iguaca Aviary, a new breeding facility to house the parrots of the Luquillo Aviary was completed in 2007 with the first breeding season in 2008. Figure 8 The breeding cages at the new facility were scattered throughout the forest understory. This management tool greatly enhanced reproduction at the facility and the continued success of the program[17].

In 2012 the Iguaca Aviary had the highest fertility rate of eggs (77%) recorded in the 40 year history of this flock. Prior to 2012, the highest egg fertility rates were in 2011 (65%) and in 2006 (60%). The fertility in this flock previously ranged between 16% and 45%. Also in 2012, 57 chicks were produced with a survival record of 70%. More recently, the 2013 breeding season finished with 66% fertility and an 86% survival record. This is the highest survival rate in the history of the Luquillo/ Iguaca Aviary captive breeding program. In the 5 years from 2009 to 2013 the Iguaca Aviary (Former Luquillo Aviary) produced more chicks than the total produced in the previous 30 years (1979 to 2008)[17].

As mentioned earlier, the general physical examinations, endoscopic examinations, hematology and plasma biochemistrie analysis of this subset of the populations of birds, at both the Luquillo/Iguaca and Rio Abajo Aviaries, revealed overall good health and condition. No indication of major infectious diseases was found. This would indicate that a high level of general husbandry, nutrition and management is being practiced at both aviaries. Captive breeding programs for reintroduction for any species are risky, complex conservation actions[18,19]. For reintroductions based on captive breeding programs, general concerns exist that these programs can 1) be costly, 2) direct funds

away from habitat restoration, 3) alter the genetic structure of future populations, 4) compromise the natural behavior of the species, and 5) have low likelihood of success[20,21]. In fact, studies reviewing the outcome of reintroduction efforts have found that ~25% are considered successful, ~25% failures, and the outcome for a majority of efforts are still undetermined[22]. However, variations in success exist among taxonomic groups[22]. Relative to our study subject, a recent publication evaluating psittacine reintroductions categorized 55% of the releases as successful, a higher rate than the broad scope taxonomic reviews[23].

Due to challenges inherent to reintroduction, scientists and managers have attempted to assess individual factors contributing to potential success or failure such as we did with the overall health and reproductive assessment. Most of the species-specific case studies have focused on factors such as restoration of native habitat, management of threats in the wild, methodology for releases, monitoring after releases, evaluation of release programs, and adaptive management of wild populations[19]. Through this study we add the importance of a proper health and reproductive assessment. When the population's growth, vital rates, and age/sex structure for the aviaries were analyzed, the captive population grew, for example from N_{1989} = 53 to N_{2015} = >500, with a positive growth rate of 1.10[24,25]. The successful growth of the Puerto Rican parrot aviary population and its ability to serve as a source for reintroduction supports the 1973 decision to build a breeding program from a small population of 13 parrots[24,25].

Disclaimers:

"The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service."

"The use of trade names, Laboratory names and described Laboratory Techniques used by the authors of this article does not imply endorsement by the United States Government".

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