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Health and Reproductive Assessment of Selected Puerto Rican Parrots (*Amazona vittata*) in Captivity

Susan Clubb, DVM, Dipl ABVP (Avian), Jafet Velez, MSc, MAR, Michael M. Garner, DVM, Dipl ACVP, Julia Zaias, DVM, and Carolyn Cray, PhD

Abstract: The Puerto Rican parrot (*Amazona vittata*) 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 a long-term reintroduction program. In 2002, 40 adult Puerto Rican parrots that had not produced viable offspring were selected for reproductive assessment at 2 aviary populations in Puerto Rico (Iguaca and Río Abajo), which are the only sources of parrots for release. The goal was to enhance reproductive potential and produce productive pairings in an attempt to augment the population growth and provide ample individuals for reintroduction. Seven Hispanolian Amazon parrots (*Amazona ventralis*) that were used as surrogate parents for the Puerto Rican parrots were also included in the study. This assessment included physical examination, endoscopic evaluation, hematologic and plasma biochemical profiles, viral screening, and hormonal assays. Results of general physical examination and hematologic and plasma biochemical testing revealed overall good health and condition of this subset of the population of Puerto Rican parrots; no major infectious diseases were found. Endoscopic examination also revealed overall good health and condition, especially of females. The apparent low fertility of male birds warrants further investigation. The findings helped to define causes of reproductive failure in the selected pairs and individual birds. New pairings resulting from the assessment helped to augment reproduction of this critically endangered species.

Key words: psittacine bird reproduction, endoscopy, avian, Puerto Rican parrot, *Amazona vittata*

Introduction

The dramatic reduction in the population of the Puerto Rican Amazon parrot (*Amazona vittata*) during the 19th century led to its designation as a critically endangered species in 1967.[1–4] The primary causes for this decline were habitat destruction and low rates of reproduction in the wild as populations were pushed out of their historic range.[1–4] Between 1972 and 1975, the US Fish and Wildlife Service began to bring some chicks and eggs from the wild into captivity to establish a captive breeding program. The first attempt to breed Puerto Rican parrots in captivity was in 1977, and the first captive hatching was in 1979.[1–4] Captive production of the Puerto Rican parrot has been instrumental in recovery of the species from only 13 individuals in the wild in 1975 to a total population of approximately 535 in 2015. The current population comprises 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, Puerto Rico.

Avian infertility can be associated with behavioral, environmental, or medical risk factors, either alone or in combination.[5,6] Behavioral causes of infertility in captive parrots are pair incompatibility, sexual inexperience, lack of early learning,

From the Rainforest Clinic for Birds, 3319 E Road, Loxahatchee, FL 33470, USA (Clubb); US Fish and Wildlife Service, Puerto Rican Parrot Recovery Program Office, PO Box 1600, Rio Grande, PR 00745, USA (Velez); Northwest ZooPath, 654 West Main St, Monroe, WA 98272, USA (Garner); University of Miami, Division of Veterinary Resources, PO Box 016960 R-289, Miami, FL 33101, USA (Zaias); and University of Miami, Miller School of Medicine, Division of Comparative Pathology, PO Box 016960 R-46, Miami, FL 33101, USA (Cray).

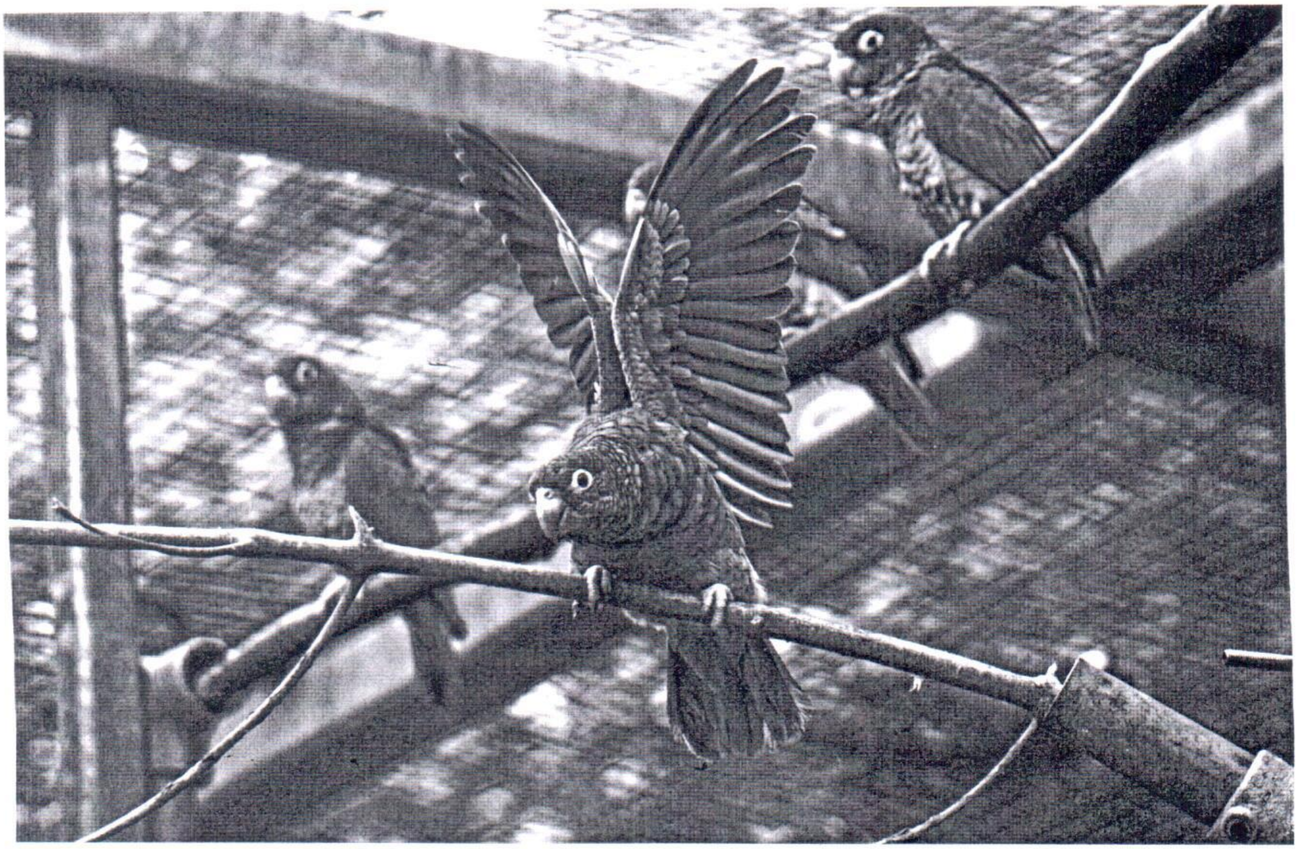


Figure 1. Puerto Rican parrots in the Iguaca aviary, Puerto Rico. This large flight cage is used for conditioning, predator avoidance training, and food recognition training of young birds in preparation for release. Photo by Pablo Torres-Baez, US Fish and Wildlife Service staff.

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 include lack of visual barriers; excessive rain, temperature, or humidity; storms or other disruptive climatic events; disturbance by pests or predators; lack of appropriate foods; loose or incorrect perches; and incorrect photoperiod, nest box or nesting materials, or enclosure design.[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, reproductive tract disease, nutritional deficiencies or excesses, systemic diseases, parasitic disease leading to malnutrition, cloacal abnormalities including excessive feathering, possible thyroid deficiency, toxins (mycotoxins, pesticides, chemicals), and musculoskeletal or neuromuscular disease (causing pain, paresis, ataxia, weakness, decreased muscle control).[5–9]

As part of a coordinated conservation program for the Puerto Rican parrot, the US Fish and Wildlife Service and the Department of Natural and Environmental Resources cooperatively manage 2 captive breeding centers for the species in Puerto Rico (Fig 1).[2–4] The purpose of this study was to investigate causes and solutions for low reproductive performance in selected captive pairs of Puerto Rican parrots at these 2 facilities, specifically low male fertility.

Materials and Methods

The study was conducted in November 2002 at the Luquillo (now Iguaca) aviary, El Yunque National Forest, and Rio Abajo aviary, Rio Abajo State Forest, Puerto Rico. A total of 47 birds (26 males : 21 females) were examined systematically. Of these birds, 38 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 (mean age, 13 years). Seven (4:3) were Hispanolian Amazon parrots (*Amazona ventralis*) that were used as surrogate parents for Puerto Rican parrot nestlings.

For physical examination, birds were anesthetized by mask induction with 5% isoflurane and maintained at 2.5%. All birds were examined for oral and cloacal papillomas. Plumage condition was evaluated by visual examination of feathers for completeness, color, and condition of the plumage.

Blood samples from each bird were collected into ethylenediaminetetraacetic acid and lithium heparin collection tubes for hematologic testing, plasma biochemical analysis, resting thyroxine (T4) levels, testosterone levels (males), and infectious disease testing.[6,8,9] All birds were tested by polymerase chain reaction (PCR) analysis of DNA extracted from whole blood for *Mycobacterium* (group-specific antigen), *Chlamydia* (group-specific antigen), avian polyomavirus, psittacine circovirus, psittacine herpes virus (Pacheco's parrot disease), and *Salmonella* (group-specific antigen) (Avian Biotech Laboratories, Tallahassee, FL, USA). Blood smears were submitted for blood parasite screening by standard microscopic examination (Avian and Wildlife Laboratory, University of Miami, Miami, FL, USA). Biochemical analysis was performed by Abaxis Vetscan analyzer (Abaxis North America, Union City, CA, USA) using whole blood. Results were compared with reference values from the International Species Information System.[10] 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 (Bronson Diagnostic Laboratory, Kissimmee, FL, USA).

For endoscopic examination, each bird was placed in right lateral recumbency with the left leg extended, and the left flank area was prepared as a surgical field. The skin and body wall were penetrated just caudal to the last rib on the left flank with a small sharp trochar, then a larger trochar inside the endoscope cannula was inserted into the initial puncture site. The cannula was removed from the trochar and a 2.2-mm rigid arthroscope (Karl Storz Endoscopy–America, El Segundo, CA, USA), was inserted for a visual examination.[11] Because the soft tissue plains shifted and sealed the puncture hole when the leg returned to a normal position, no closure of the puncture site was required.

In females, ovaries were assessed and scored on a scale of 1 to 4, from minimum to maximum, for the following parameters: stroma size (mass of small follicles); follicle number; follicle size; amount of retained yolk material, indicating the presence of postovulatory 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.

In males, testicular morphology was subjectively evaluated and scored on a scale of 1 to 4 for the following parameters: relative size, small to large; vascularization and hyperemia, normal white to red or hyperemic; texture of the serosal surface, normal smooth to 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 also assessed to evaluate if the testes were small or the adrenal gland was enlarged. This was expressed in a ratio, with 1:1 indicating the adrenal glands are the same size as the testes.

Paired skin and feather biopsies were performed in 7 birds with obvious feather abnormalities associated with feather plucking. Paired biopsies allowed comparisons between affected (plucked) and unaffected areas.[12] Skin biopsy samples were fixed in formalin, processed routinely, sectioned at 3 levels at 5-µm thickness and stained with hematoxylin and eosin for histologic examination.[12] Testicular biopsy was done in 4 birds with atrophic testicles. Normal testicles were not biopsied because of the risk of damage in testes that are active and hypertrophied.[8,13]

Data were analyzed for descriptive statistics with Excel software (Microsoft Corp, Redmond, WA, USA).

Results

General body condition was judged by physical examination to be excellent in 32 birds (68%), good in 4 birds (9%), fair in 8 birds (17%), and poor in 3 birds (6%). Body condition (scale 1–5) assessed by keel score was ideal (3) for 40 birds. Only 2 birds were considered slightly overweight, and 5 were slightly underweight. Twenty-nine birds (62%) had excellent plumage condition, 8 (17%) had good to fair plumage, and 10 (21%) showed feather-damaging behavior, 2 of which were plucked on the head by their mates. The mean body weight was 297 g (range, 239–399 g) for all birds, 292 g (range, 239–399 g) for females, and 302 g (range, 245–398) for males.

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 examination.

This bird died 2 years later, and no evidence of papillomas was found at necropsy.

Results of hematologic testing and plasma glucose, total protein, albumin, globulin, AST (in birds tested), and uric acid concentrations were within reference intervals in all birds. Concentrations of creatine kinase (CK) were available for 32 birds and ranged from 183 to 4589 U/L; levels in 22 birds were higher than the reference intervals for Amazon parrots (55–345 U/L; Avian and Wildlife Laboratory, University of Miami). Results of infectious disease assays were negative for all birds. All blood samples were negative for blood parasites.

Thyroxine (T4) analysis was performed in 37 Puerto Rican parrots. The range of T4 values was 0.07–1.8 μg/dL (mean ± SD, 0.5 ± 0.4 μg/dL; reference intervals for Amazon parrots, 0.1–1.1 μg/dL [Avian and Wildlife Laboratory, University of Miami]). The range of T4 values in females (13 birds) was 0.17–1.16 μg/dL (mean, 0.47 μg/dL) and in males (24 birds), 0.07–1.8 μg/dL (mean, 0.52 μg/dL). Values in 2 male birds (ages 4 and 17 years) fell below this range. Four birds, 1 female (age 26 years) and 3 males (ages 3, 16, and 27 years) had T4 values above the reference interval. Because the birds were evaluated at different times of the day, diurnal variation might account for some of this variation. No data were 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 normal values except in 1 bird that was 34 years old. Reported testosterone values for 10 avian species, almost without exception, exceeded 0.2 μg/mL.[8] Similar values, typically greater than 0.2 μg/mL, were reported for 6 avian species.[14] Testosterone levels are noted to be very variable seasonally. Interlab and intralab variations are also well documented.

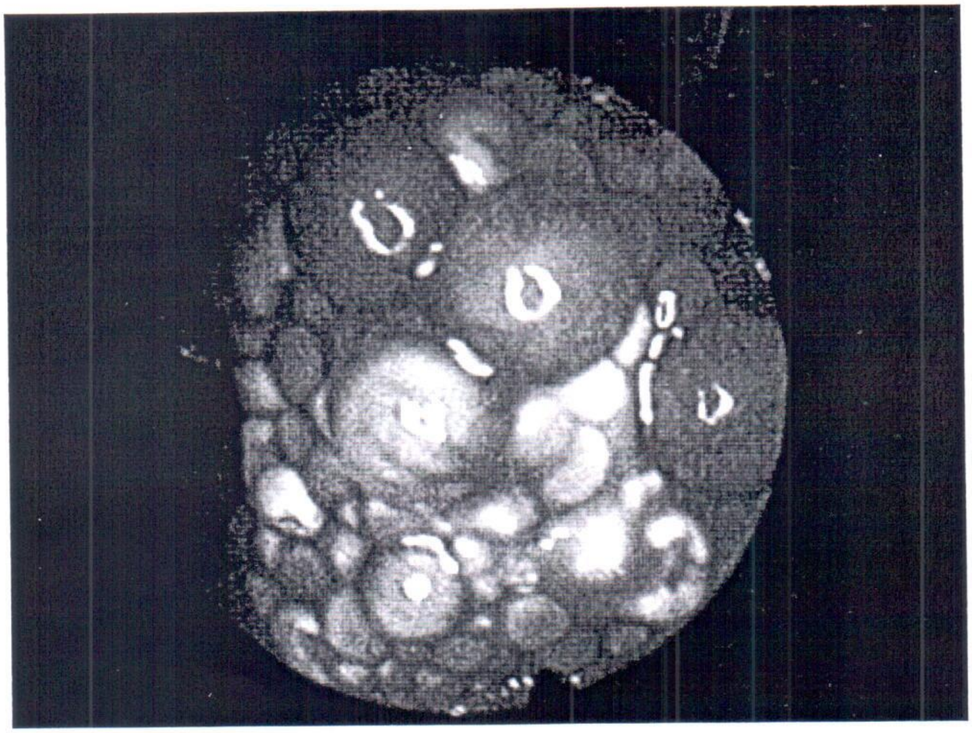
On endoscopic examination, the air sacs of 44 birds were clear and appeared normal. Of the remaining 3 birds, 1 bird had cloudy air sacs, 1 had lesions suggestive of previous aspergillosis, and in 1 bird the air sacs were very vascular with floating debris. This bird was isolated; samples of the air sacs submitted for culture were positive for *Pseudomonas aeruginosa,* and the bird subsequently was treated for the infection. Examination of the coelomic organs were normal in 42 birds with findings 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 (46%) birds, but in several birds it appeared to become reactive to the touch of the endoscope and visibly became more distended during the examination. The birds were fasted before examination, so this enlargement was unlikely to be due to engorgement with food. The cause for this proventricular enlargement was unknown; however, this observation of proventricular enlargement was very subjective and may be an idiosyncrasy of this species. Proventricular enlargement did not seem to correlate with any specific age group (mean, 13 years; range, 1–28 years). General body and feather condition of birds with proventricular enlargement appeared similar to that of the total population. The gonadal condition roughly correlated with proventricular enlargement: the ovaries appeared subjectively more active in females with proventricular enlargement than in females without enlargement.

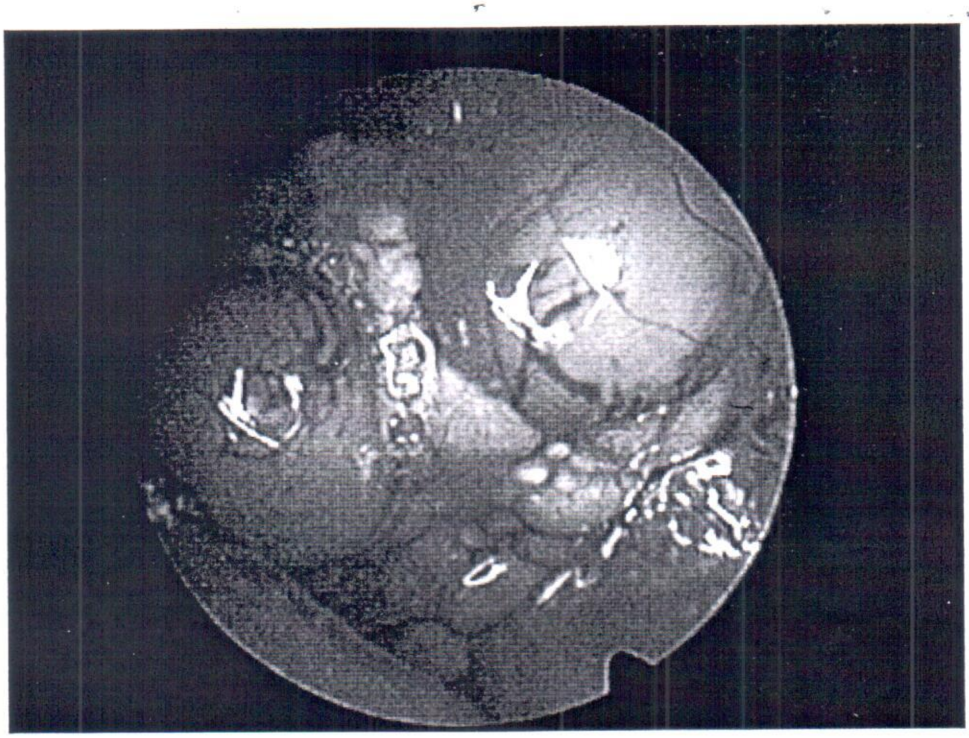
In 4 birds, variable yellow foci were observed in the liver parenchyma (multifocal). No liver biopsies were done. Of these birds, 1 was male, 3 were female, mean body weight was 310 g (above average for population), and ages were 4–17 years. The 3 females had very active follicular development, indicating that these foci may have been fatty and possibly associated with mobilization of reserves in association with yolk formation. Hematologic and plasma biochemical values in these birds did not suggest infectious disease.

The ovarian stroma and its mass of various-sized follicles appeared normal in 17 females and small in 4 females (ages 1 year, 14 years, and 2 birds, 29 years) (Fig 2). In one 29-year-old bird, the small stroma was attributed to depleted embryonic follicular sites and exhaustion of ovulation potential. For number of visible ovarian follicles, 2 birds were scored as 1+ (both age 29 years) with very few visible ovarian follicles; 5 birds as 2+ (mean age, 14 years; range, 3–26 years); 6 birds as 3+ (mean age, 9 years; range, 1–14 years); and 7 birds as 4+ (mean age, 12 years; range, 4–23 years), with large numbers of follicles spread evenly across the stroma. The 2 birds aged 29 years appeared to have partially exhausted their reproductive potential; however, the breeding-age birds showed good potential for active egg production.

For ovarian follicle size in the Puerto Rican parrot females, 4 birds were scored 1+ (mean age, 19 year; range, 3–29 years), with no enlarged follicles; 6 birds as 2+ (mean age, 9 years; range, 1–19 years); 7 birds as 3+ (mean age, 10 years; range, 4–23 years); and 1 bird as 4+ (age 8 years), with many large follicles (Fig 3). Because the active



'igure 2. Small- and medium-sized follicles are seen via endoscopy in this ovary of a sexually active female Puerto Rican parrot. Yellow material probably represents postovulatory follicles. This female would have excellent eproductive potential.



'igure 3. This very active ovary of a Puerto Rican parrot examined via endoscopy shows a mixture of large-, medium-, ınd small-sized follicles as well as postovulatory follicles (yellow) and fibrotic follicles (white).

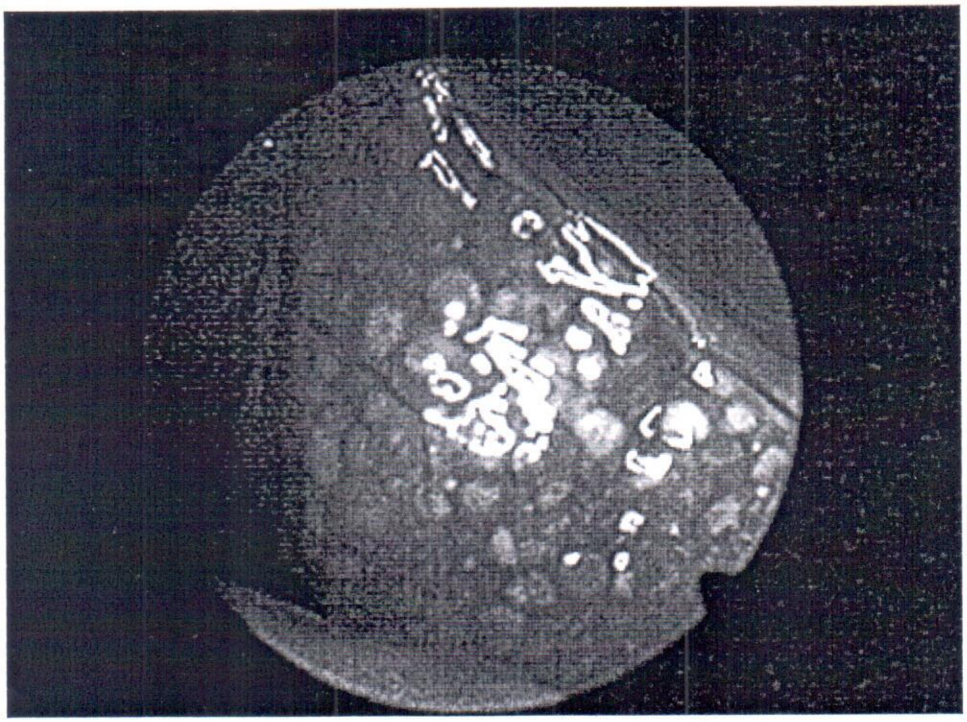


Figure 4. Thickened membranes partially obscure this ovary of a Puerto Rican parrot examined via endoscopy. This may indicate an inflammatory process in the ovary.

period of egg laying for the Puerto Rican parrot is in the spring, this evaluation in November indicated excellent reproductive potential for females in this population. Only 1 bird showed very large follicles; however, this would not be unusual in the nonbreeding season.

The presence of retained yolk material, presumed to be postovulatory follicles or atretic follicles, was evaluated in the 18 female Puerto Rican parrots. No yolk material was seen in 5 birds (mean age, 17 years; range, 1–29 years); 2 birds had 1+ (ages 7 and 14 years), with very little retained yolk material; 8 birds had 2+ (mean age, 15 years; range, 7–26 years); 2 birds had 3+ (ages 8 and 11 years); and 1 bird (age 29 years) showed white scarring.

Most females had little oviduct hypertrophy, consistent with oviductal involution when birds are not in active reproduction in the nonbreeding season.

Of the total 21 female parrots, most birds (18) had no visible ovarian membranes. Thickened membranes were assessed as 2+ in 2 birds (ages 26 and 29 years) and 4+ in 1 bird (age 23 years), with membranes obscuring visualization of the ovaries (Fig 4). Opacity and thickening of membranes surrounding the ovaries can be associated with infection or nonspecific inflammation or may indicate advanced age.

Hypertrophy of the vent tissue, a common finding in sexually active females, indicates hormonal conditions conducive to egg production and is a seasonal physiologic change in reproductively active females. This change was observed in 10 females (mean age, 10 years; range, 3–20 years). Surprisingly, hypertrophy and flaccidity of the vent seemed much more indicative of reproductive readiness than follicular enlargement observed by direct endoscopic examination of the ovaries.

The ovaries in 2 older birds (ages 29 and 20 years) were biopsied. One bird (age 29 years) had a moderate number of follicles containing dense and white material (Fig 5). Results of histologic examination in this bird revealed proteinaceous material, possibly granulomatous, with inflammation and fibrosis consistent with a senescent ovary. In the 20-year-old bird, the material was more consistent with a postovulatory follicle and mild inflammation and thickened membranes overlying the ovary. It was not determined if the accumulation of this material is consistent with a pathologic change or rather is a normal physiologic finding in multiparous females.

Testes were evaluated in all males, and testicular biopsies were attempted in 4 birds with testes that

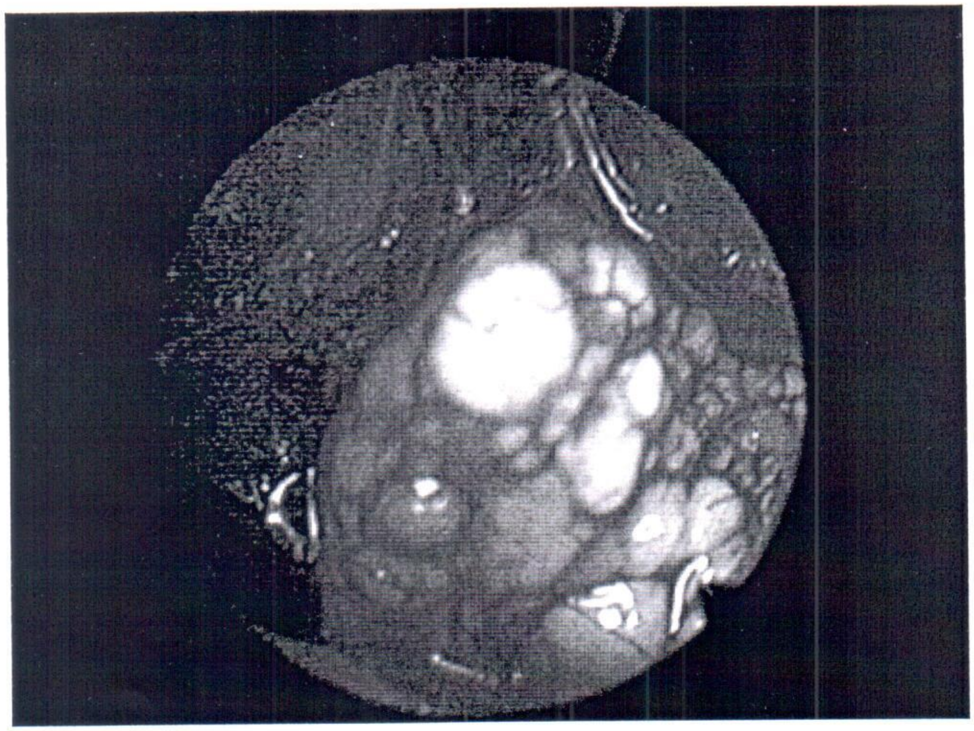


Figure 5. A 29-year-old Puerto Rican parrot hen examined via endoscopy. Note retained material appeared white rather than the normal creamy or yellowish material. On biopsy, this material was identified as fibrotic tissue. This bird probably has little potential for further egg production and could be considered senescent.

appeared to be abnormal or atrophic. Biopsy was successful in only 2 birds. Two testicular biopsy samples had nicely preserved testicular tubules, neither with mature sperm. Both showed mild capsular chronic inflammation and mild (<5%) fibrosis (Fig 6).

Testicular size was subjectively estimated because the degree of seasonal variation that would be expected is not known. Breeding birds are not typically evaluated during the breeding season as this disruption would likely result in subsequent loss of production. Despite seasonal variation, relative testicular size seemed to correlate with expected changes associated with age (immature birds and advanced age). An unexpected finding was a 34-year-old male with very large, active testes. Of the remaining 25 males, testicular size (small to large) ranged from 4 birds with 1+, (mean age, 9 years; range, 2–27 years); 12 birds with 2+ (mean age, 11 years; range, 4–18 years), 7 birds with 3+ (mean age, 15 years; range, 8–23 years), and 2 birds with 4+ (ages 18 and 34 years).

Vascularization and hyperemia of the testes was subjectively assessed. Orchitis is often evident by visual hyperemia of the testes and is a common finding in male Amazon parrots.[5–8,13] Twenty-five males had normal-colored testes that indicated a relatively low level of orchitis, and only 1 bird had 4+ hyperemia.

The texture of the testicular surface/presence of cyst-like structures or irregularities was evaluated. Cyst-like structures can indicate tubular abnormalities or dilations of tubules, which may or may not affect fertility.[5,13] Of the 26 males, 20 birds had normal, smooth surface texture, whereas 6 birds had slight irregularities in texture or surface (age 3–16 years).

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 a sequela to chronic inflammation as indicated by opaque irregular appearance of the tissue.[13] Increased testicular density was seen in 3 birds (mean age, 16 years; range, 5–27 years). In another bird (4 years), the testes appeared very hard and dense. This is a very uncommon finding in a young bird, and 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. No abnormalities were observed in any birds.

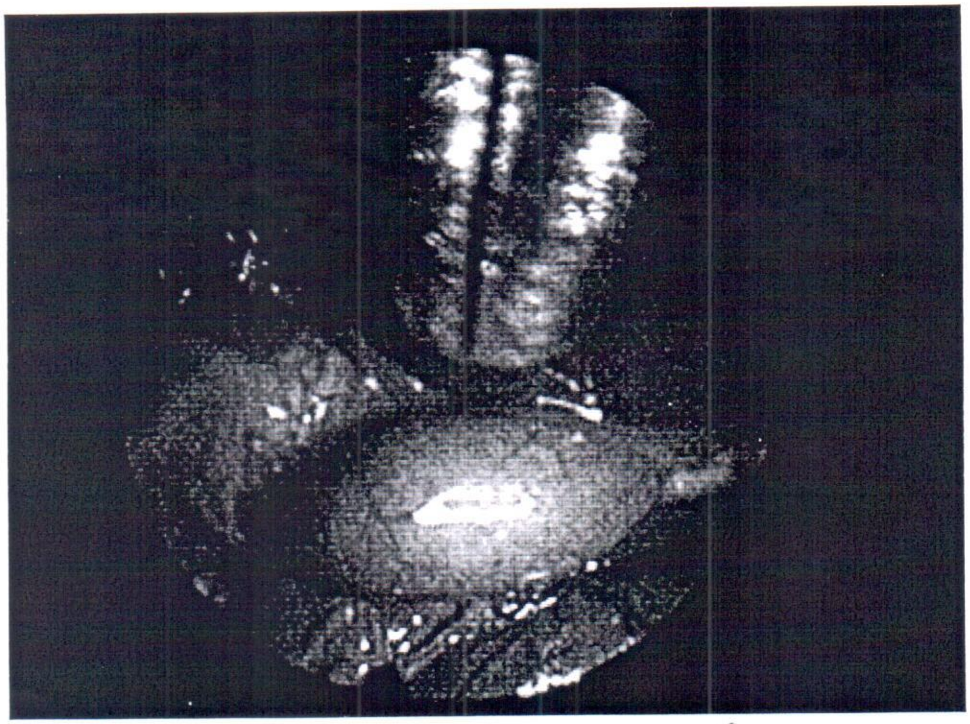


Figure 6. Biopsy forceps approach a small, dense testicle of a male Puerto Rican parrot. This bird had fibrosis believed to be secondary to chronic orchitis. The adrenal : testicular size ratio in this bird is 1:1.

An adrenal : testicular size (A : T) ratio of 1:1 was found in 8 Puerto Rican parrots (mean age, 12 years; range, 2–28 years). An A : T ratio of 1:2 was found in 5 birds (mean age, 9.4 years; range, 4–16 years); 1:3 in 4 birds (mean age, 14.5 years; range, 8–18 years); 1:4 in 3 birds (mean age, 14.5 years; range, 8–23 years); and a ratio of 1:5 or 1:6, indicating very large testes, was found in 3 birds (mean age, 21 years; range, 11–34 years) (Fig 7). A ratio of 1:1 is considered normal in immature birds, but in adults, this may indicate stress-related suppression of testicular function or adrenal hyperplasia in response to chronic stress (Fig 8).

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 birds had no visual opacity or hypertrophy of peritesticular membranes. Only 1 bird had a 3+ score.

In all 7 birds that had paired skin and feather biopsy, histopathologic examination revealed perivascular dermatitis and possible hypersensitivity, which may have resulted in the feather plucking. These birds were 4 males (mean age, 25 years; range, 15–34 years) and 3 females (mean age, 15 years; range, 14–20 years).

Discussion

Results of general physical examination and hematologic and plasma biochemical testing revealed overall good health and condition of this subset of the population of Puerto Rican parrots, at both the Luquillo (renamed Iguaca in 2007) and Rio Abajo aviaries. No major infectious diseases were found. These results 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 females showed good overall reproductive potential, consistent with maturity during the nonbreeding season. Birds in their late 20s might be considered functionally senescent with low expectation of reproductive success, possibly indicating that 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. This bird was caught in

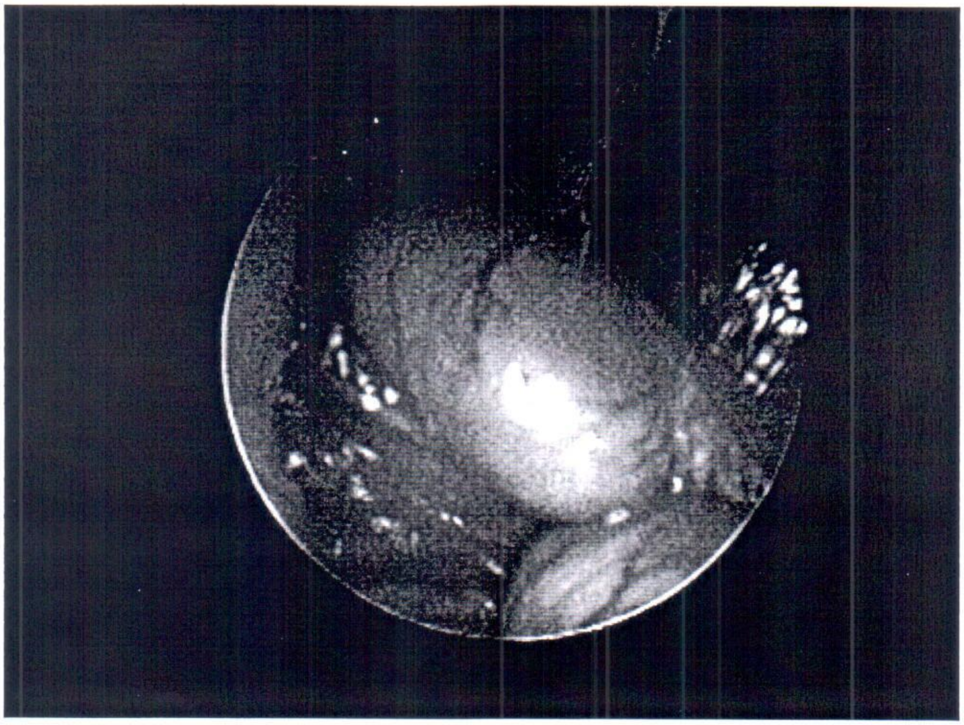


Figure 7. The large active testicle of a male Puerto Rican parrot examined via endoscopy. The testicle obscures the adrenal gland.

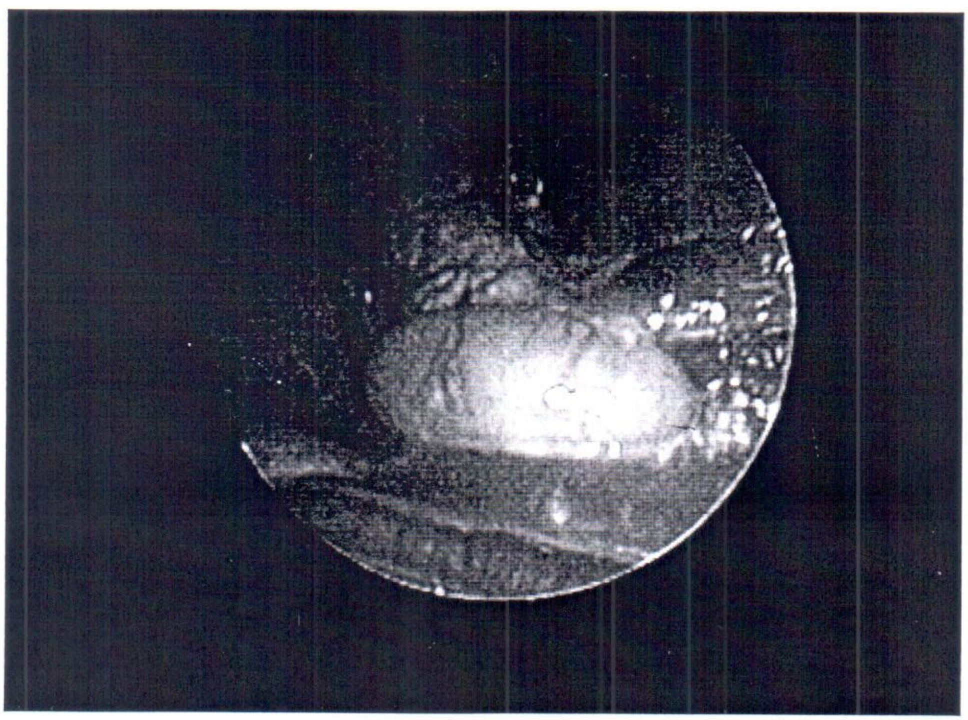


Figure 8. The enlarged adrenal gland in this male Puerto Rican parrot evaluated via endoscopy is roughly equivalent in size to the testicle. This would be an adrenal : testicular size ratio of 1:1, which could indicate a stress response and subsequent poor reproductive performance.

1973, estimated to have hatched in 1968, and died in 2008 (J. V., unpublished data, 2008).

Retained yolk in postovulatory sites indicates previous ovulation.[9] This material often accumulates in older birds (S. C., unpublished data, 2002).

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. An increase in the size of the adrenal gland in relation to the testes can also be a subjective indication of stress. Stress and the subsequent endogenous glucocorticoids theoretically can suppress seasonal testicular hypertrophy. Therefore, in visually comparing the adrenal and testicular size, if the adrenal gland is hypertrophied and the testes are small, this may indicate stress-related suppression of testicular function. This comparison is very subjective because the testes cannot be directly measured as is done in reproductive assessment in mammals; however, this visual judgment gives a basis for comparison of males. From our results, the greatest reproductive potential for males appears to be during the teen years, which is consistent with findings in some other psittacine species.[7] The very active 34-year-old bird may be an indication of prolonged reproductive potential or might represent an outlier of individual variation. Despite the apparent fertility of this male, only 1 offspring has been produced.

Testicular biopsy was attempted in only 4 birds. Testicular biopsy poses some risk of damage in testes that are active and hypertrophied.[8,13] Leakage of tissue could theoretically result in an autoimmune reaction against the bird's own testicular tissue.[8,13] In our experience, testes that are atrophic do not leak after biopsy.[13]

Plasma testosterone levels have been reported to vary with age, season, 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 the preliminary findings of this study appear to be significant and certainly warrant further investigation. These low testosterone levels could reflect the nonbreeding condition of the parrots in the fall. A logical next step in this investigation would be to sample plasma testosterone levels in males that are captured for any reason during the breeding season. Special attention should be placed on birds showing aggressive behavior.

In 7 birds, skin biopsy revealed dermatitis and possible hypersensitivity. 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 depend on chronicity of the condition and the degree of irreversible damage to feather follicles. No correlation of age with T4 level or with hypersensitivity dermatitis was apparent.

The apparent enlargement of the proventriculus and elevated plasma CK levels in many of the birds lead to a concern about the potential presence of proventricular dilatation disease in the population. However, this is possibly a normal variation in the species and does not indicate any underlying disease process, but it warrants further investigation. One explanation for the proventricular enlargement may be the dose-dependent effect of isoflurane on smooth and skeletal muscle relaxation during endoscopy. Histopathology is performed on every bird that dies at these aviaries, and proventricular dilatation disease has never been found in this population. The lack of previous cases of proventricular dilatation disease supports the assumption that these findings do not indicate this disease being present in this population. The yellow foci in the liver, as detected endoscopically, were also of mild concern, despite the negative PCR for mycobacterium in all birds tested. Targeted hepatic biopsy would provide definitive information.

No obvious correlation was found between enlarged proventriculus on endoscopy and high CK levels. High CK levels appeared to be independent of sex or age. Reported reasons for increased plasma CK levels include blunt trauma, capture myopathy, exertion, feather-picking, furazolidone cardiotoxicosis, irritating injections, nutritional myopathy, self-mutilation, starvation, or surgery.[15] Proventricular dilatation disease can also result in high plasma CK levels.[16] In this study, blood samples were 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 in CK levels. A review of medical records since this study demonstrated that high CK levels are very common nonspecific findings 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 a causative factor in this population. However, low testosterone levels are of concern, even during the nonbreeding season. This correlated with a relative high percentage of birds with small testes. These findings warrant further investigation. Thyroid dysfunction did not appear to be involved, as T4 levels were generally within the normal range.

In a study on management of Puerto Rican parrots, the theory of psychological castration of males has been proposed (J. V., unpublished data, 1987). Psychological castration results because of males being maintained in adjacent cages without a sense of secure territorial boundaries between pairs. Visual barriers were installed in the aviaries in an attempt to address this potential problem. Pairing young males with older egg-laying females was also recommended. One breeder of Amazon parrots suggests that species with red frontal coloration and red wing speculums use these color patches in sexual displays both with females and other males (H. J. Voren, oral communication, November 2002). Therefore, housing breeding males that have red frontal coloration in close proximity to each other may suppress reproduction. Keeping pairs of other Amazon species without red frontal coloration in adjacent cages may reduce this phenomenon. This would appear to correlate well with the theory of psychological castration.[17] The suspicion of low male virility in the apparent absence of disease supports behavioral or physiological factors as being important. Theoretically, pheromonal 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 Puerto Rican parrots 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 supports 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 include larger cages, visual barriers, and vegetation between cages to reduce psychological or possibly pheromonal 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 nonbreeding season is another management tool that has been shown to enhance breeding success of Amazon parrots. A similarly colored and also endangered species, the red-browed Amazon parrot (*Amazona rhodocorytha*), showed greatly enhanced breeding success in years subsequent to flocking in the nonbreeding season at Loro Parque, Tenerife, Spain (S. C., unpublished data, 2005). 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 Puerto Rican parrots 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 provide further psychological and physiologic enrichment for breeding males at the time they are moved into the new breeding enclosures.

After 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 Puerto Rican parrots. As a result of this study, 3 nonproducing pairs were repaired, and 5 new pairs were formed with positive results. Between 2003 and 2005, 32 fertile eggs and 22 hatchlings were produced as a direct result of these new pairings. This significantly increased the captive population of Puerto Rican parrots 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. 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. Before 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]

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 approximately 25% are considered successful, 25% are failures, and the outcome for most efforts are still undetermined.[21] However, success varies among taxonomic groups.[22] Relative to our study subject, a recent publication evaluating psittacine bird reintroductions categorized 55% of the releases as successful, a higher rate than that in the broad-scope taxonomic reviews.[23]

Because of 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 of the Puerto Rican parrots. 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 release, monitoring after release, evaluation of release programs, and adaptive management of wild populations.[19] Through this study we add the importance of a thorough health and reproductive assessment. When the growth, vital rates, and age/sex structure of the populations 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 of the species supports the 1973 decision to build a breeding program from a small population of 13 parrots.[24,25]

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