Managing the World's Largest Population of Spix's Macaws (Cyanopsitta spixii)

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Abstract
The Al Wabra Wildlife Preservation (AWWP) is currently home to 50 individuals of the world's most threatened parrot, the Spix's Macaw (*Cyanopsitta spixii*), representing 67% of the birds listed in the international studbook for the official captive breeding program. As a consequence of assembling a large flock from diverse sources, a high incidence of disease was initially found to be present within the AWWP population, constituting a wide variety of psittacine pathogens. In an attempt to combat the high incidence of disease, AWWP has invested in an extensive health management program. Valuable data on pathogenesis, transmission, clinical presentation, diagnostics and treatment have been collected. The extremely low level of heterozygosity in the captive population as a whole, as a result of inbreeding and over-representation of one bloodline, is an additional challenge in the management of AWWP's Spix's Macaws. It seems, the lack of genetic diversity has already manifested itself in the form of infertility, late maturation, embryonic mortality and chick deformities. Despite all the challenges and setbacks the breeding program is progressing, with recruitment now significantly exceeding mortality.

Introduction to Spix's Macaws at AWWP
The Al Wabra Wildlife Preservation (AWWP) is the private wildlife collection of His Excellency Sheikh Saoud Bin Mohd. Bin Ali Al-Thani. Located in the barren centre of Qatar, AWWP has a primary focus on the propagation of rare and threatened species, including the Spix's Macaw (*Cyanopsitta spixii*). The Spix's Macaw is often considered to be one of the world's most threatened birds and has not been observed in the wild since the last known individual disappeared in October 2000. Currently, there are also 78 birds in the official captive breeding program supported by the Brazilian Governments branch for natural heritage and sustainability— INSTITUTO BRASILEIRO DO MEIO AMBIENTE E DOS RECURSOS NATURAIS RENOVÁVEIS (IBAMA), as listed in the international studbook. At present, AWWP maintains 67% (n = 50) of these captive birds.

In the same year that the last known Spix's Macaw disappeared from the wild, a new chapter in the history of the species was being written with AWWP receiving its first two pairs of Spix's Macaws from Birds International Incorporated (BII) in the Philippines. Subsequently, in 2002, AWWP received one male and three female birds from Swiss aviculturist, Roland Messer.

Following a successful year in 1999 when BII produced 10 offspring, the next four years only yielded another 3 birds. Additionally, avian disease was having an impact on their population. Furthermore, at that time Avian Influenza was sweeping through Asia and BII is located in very close proximity to a chicken farm. These factors played an important role in BII's decision to transfer ownership of the birds to AWWP. Accordingly, AWWP received the entire remaining Philippine population of 25 Spix's Macaws, in four shipments between the end of 2003 and early 2004. AWWP was aware that there was a high incidence of disease and other serious health issues within the new stock and

similarly it was acknowledged that many of the birds coming to AWWP might never reproduce.

Around the same time that the Philippine Spix's Macaws were arriving in Qatar, AWWP also took over the management of Roland Messer's population, following allegations from the Swiss authorities against Messer regarding insufficient husbandry standards. Shortly afterwards, Messer transferred ownership of 11 of his 13 birds to AWWP, whilst retaining ownership of the only breeding pair in the group. Within approximately two months of the transfer of these birds to AWWP's ownership, it became apparent that all 11 of the birds now under the ownership of AWWP suffered from psychological or physiological conditions of some kind, including PDD in one bird.

In the interest of bio-security, AWWP continued to manage the Swiss population separately from the Qatar population. However, in late 2005, the situation in Switzerland became unworkable due to staffing problems as well as a failed attempt to steal the birds. We had been planning to transfer the birds to the United Kingdom, but the plans relied on the cooperation and prompt action of the British authorities. With winter fast approaching, swift action was necessary but not forthcoming so the only viable option was to bring the birds to Qatar. At AWWP a temporary quarantine facility was constructed, containing six indoor aviaries. This was the home of the birds for next 6-12 months before they were progressively integrated into the AWWP breeding facility.

Spix's Macaw Health Management

Due to the many veterinary issues within the newly acquired Spix's Macaw flock as well as the conservation importance of the species, AWWP developed and implemented a rigorous veterinary program. Whilst maintaining the birds in isolation, regular health screening was carried out, with consultation from parrot specialist veterinarians Dr. Marcellus Burkle and Dr. Susan Clubb. At each health check, diagnostic techniques included complete blood counts (CBC), blood chemistry, choanal and cloacal cultures, radiography and endoscopy. Amplifying target nucleic acid via the polymerase chain reaction technique (PCR) and serology was used to test for viral diseases and crop biopsies for Proventriculus Dilation Disease (PDD).

The health checks revealed that both the Swiss and Philippine populations had been exposed to PDD, Avian Polyoma Virus (APV), Avian Paramyxo Virus (APMV), Avian Herpes Virus (AHV) and Psittacosis (Avian Chlamydia). We also learnt that as a species they seem to be particularly susceptible to the bacterium *Pseudomonas aeruginosa*, as evidenced from repeated identification of the bacteria from healthy birds, in the absence of any clinical signs. Endoscopy revealed that our Spix's Macaws appear to have unusual air-sac physiology, with certain air-sac walls appearing in random locations and occasionally missing all together in some individuals.

The former Philippine population, whilst outwardly in better health than the Swiss population, has more occult veterinary problems. The air-sacs of the Philippine birds were very cloudy and many of them exhibited an anthracosis of the lungs, the likelihood of which was higher in older birds. Both conditions are most likely associated with heavy air pollution around Manila where BII is located. Subsequently we have discovered that these conditions can improve over time but it is a very slow recovery process. Of all the former Philippine birds, the three with the clearest

air-sacs are of the first four received in 2000. Unfortunately, endoscopy was not performed on any of AWWP's Spix's Macaws until 2004, so we cannot determine the status of the air-sacs when the birds first arrived. In general however, on the basis of data from birds endoscoped multiple times over the past three years, we can ascertain that the condition is slowly improving.

Another interesting difference between the two populations is the eye color; with the Philippine population all having extremely dark irises whilst all the Swiss birds have completely white irises. In the case of the eyes, we suspect that the dark iris is caused by heavy metal accumulation, as certain heavy metals are known to deposit in the eyes and are likely to be found in high concentrations in the polluted air engulfing the highly industrialized city of Manila. The discoloration of the iris takes much longer to fade and is variable according to each individual. Some bird's eyes do not appear to be fading at all, while others have undergone significant fading. It is our understanding that heavy metals are very difficult to purge from the body, so it is possible that none of the former Philippine birds will ever have the same white irises as the former Swiss birds

Proventriculus Dilation Disease (PDD) in Spix's Macaws at AWWP

Of the eight Spix's Macaws that have died since we lost our first bird in 2003, PDD has been responsible for five of these deaths. Three of these PDD-related deaths occurred in 2004, in birds we had accepted knowing that they were not healthy and possibly suffering from PDD. There is still much to learn about PDD, and by accepting these birds AWWP has been provided with valuable experience in recognizing the symptoms, understanding the pathogenesis, and attempting treatment of the disease, which could help to save other birds from PDD in the future.

It has been noted at AWWP that Spix's Macaws affected with PDD do not develop the typical massively dilated proventriculus or pass undigested seeds in the droppings as is typical for the disease. Instead, they suffer progressive damage to the Central Nervous System (CNS) and PDD is often only confirmed when post mortem samples from the CNS, adrenals, heart and digestive organs are sent for histopathological examination. Another possible link that is being explored is the relationship between Avian Paramyxo Virus (APMV-1) and PDD. A growing body of evidence suggests that an APMV-1 titer will be found in bird collections maintaining PDD-positive birds and can therefore be used as a preliminary indicator (GRUND 1999).

Since 2004, 73 crop biopsies have been taken from 46 individual Spix's Macaws at Al Wabra, with several individuals having had as many as three biopsies during this period (see table 1). A crop biopsy is considered to have a sensitivity of between 60-70% for detecting the changes in nerve cells caused by PDD. A positive test for PDD is said to be definitive but a negative (no obvious lesions) test is no guarantee that a bird is free of the disease even if they present healthy. The third and most frustrating result is when a bird presents sub-clinical symptoms, which raise suspicions for PDD (indicated by signs of lymphoplasmacytic inflammation surrounding ganglia but not within the ganglia). With such an important species as the Spix's Macaw, they have to be managed as though they are PDD positive in order to mitigate the risk for the rest of the breeding population. Of the five PDD-related deaths at Al Wabra, two birds tested positive and three suspicious for the disease. Of these five dead birds, one also tested

positive for APMV particles by scanning electron microscope in the faeces, whilst one other bird had an antibody titer for the APMV-1 virus.

Another bird to test positive for PDD by crop biopsy did so in 2004, but ever since has had no obvious lesions (indicating a negative result) from biopsies in 2006 and 2007. Radiographs show that although the bird does have an enlarged proventriculus in comparison to other Spix's Macaws, it is not significantly enlarged compared to those seen in species suffering from the classic proventriculus dilation syndrome and, moreover, remained unchanged over the three years. This bird has never been clinically ill during this period and never passed undigested food in its faecal nor shown any signs of CNS damage. This strongly suggests that this bird has maybe overcome the infection. Interestingly, its virology test results also show that the bird was positive for APMV-1 antibodies in 2006 but negative thereafter in 2007.

Table 1: 73 crop biopsies have been taken from 46 individual Spix's Macaws (some individuals having had several biopsies during this period)

PDD	crop biopsy results	PDD	birds still alive
Positive	3	2	1
Suspicious	14	3	9
Negative	56	0	31
Total (since 2004)	**73**	**5**	**41**

Note: None of the 13 Spix's Macaws bred since 2005 have had crop biopsies.

Of the nine living birds to test suspicious for PDD, seven have exhibited no obvious lesions in subsequent crop biopsies, one bird has tested suspicious twice thereafter, and one bird was suspicious in the most recent crop biopsy and will be tested again late in 2007.

Notes on other psittacine diseases at AWWP

Avian Polyoma Virus (APV) so far does not appear to be a big problem for Spix's Macaws at Al Wabra. We have detected APV anti-bodies in 26 out of 50 Spix's Macaws tested and have detected APV-antigens in the blood of two out of 46 individuals tested by PCR. Of these two individuals, one young Spix's tested positive for an antigen, whereas it did not have any antibodies to APV and in subsequent tests, it tested negative for both the antigen and antibody. It seems probable that the one isolated APV-antigen result could have been from possible contamination of the sample. The other bird to test positive for APV antigens was an adult bird that died a few months later of PDD.

A pattern that emerged over the years of testing for APV-antibodies is that some Spix's Macaws are consistently positive for the antibodies whilst others are consistently negative. We have not been able to connect APV to any deaths or illnesses in Spix's Macaws; however, the disease is known to be sub-clinical in adult large psittacine species while posing a significant threat of acute disease and mortality in chicks and fledglings.

Avian Herpes Virus (AHV) is one of the most successful of all viruses, with strains having been isolated from most species of birds. Most AHV strains are host specific and ubiquitous within a population. Host adapted strains generally cause mild, sub-clinical, latent infections in their natural host; however, severe, often life-threatening disease, such as Pacheco's, can occur when a non-

adapted Herpes Virus enters a new host (Ritchie, B. 1995). Since 2004, 57 of the 61 Spix's Macaws that have been in the care of Al Wabra have been tested for AHV antibodies, including 47 of Al Wabra's current stock. In total 146 tests have been done, resulting in 13 positive AHV-antibody results from 10 individuals, three of which have tested positive twice. Nine of the 10 birds have tested negative in tests done before or after the positive finding.

Only one Al Wabra Spix's Macaw has ever tested positive for AHV-antigen and that was a young female bird that we bred in 2005. This result was particularly interestingly because this bird was hand-reared from the egg, indicating possible vertical transmission. In addition, although the dam tested positive for AHV- antibodies, she never tested positive for AHV-antigens. The only other access the young bird had to other birds was to two other young Spix's Macaws that were also reared from the egg but tested negative for AHV-antigens or antibodies when tested at the same time. The antigen positive female bird had no detectable AHV-antibodies, so we decided to test her and her two companions again six weeks later. The result was that the previously antigen-positive bird was no longer positive for antigen, but now had AHV-antibodies. Meanwhile, one of its companions now had AHV-antibodies, which suggests that these birds were being exposed to the virus. Fortunately, neither of these two birds nor, for that matter, any other Spix's Macaws at AWWP has shown any serious clinical signs that can be directly linked to an AHV infection.

AWWP has never experienced a clinical case of Psittacosis in Spix's Macaws or any other of their bird species. During health checks, Chlamydophila swabs are taken from the choana and cloaca to test for antigen and blood serum is used to detect antibodies. 82 Chlamydophila test swabs have been taken from 56 Spix's Macaws and none have been positive for antigens. 67 serum samples from 48 Spix's Macaws have been tested for antibodies and 20 have tested positive. None of the 20 antibody positive birds have been positive in later tests and there has not been a positive result since January 2005.

Finally, AWWP also tests for Avian Tuberculosis (serovar 1-3) (ATB) as part of our health program. These tests commenced in late 2006, as it was included as a required test in newly established health guidelines laid down during Spix's and Lear's Macaw committee meetings held in November 2006. AWWP has never had a case of clinical ATB so it was no surprise that none of the 45 birds tested to date have tested positive for the disease.

Spix's Macaw Faecal Bacteriology

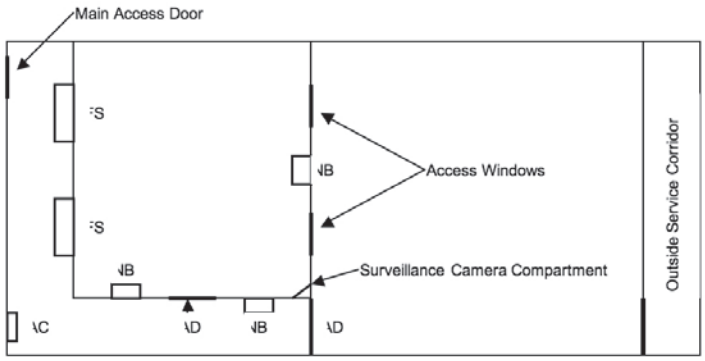
In an effort to monitor the bird's health, a monitoring of monthly faecal samples was established to examine bacterial flora changes and parasite loads. This is because finding gram-negative bacteria is an abnormal finding in most psittacines and therefore their detection can act as an early indicator for illness in the Spix's Macaws. Although most of the Spix's Macaws that arrived from the Philippines were initially infested with Cestodes (Tape Worm) a thorough de-worming program took care of it. What we have discovered since sampling began back in 2004 is that there appears to be a lack of consistency with the bacteriology results. According to Ritchie et al (1994) faeces from a healthy parrot should contain approximately 100-200 gram-positive bacteria per microscopic field of view under x1000 magnification and no gram-negative bacteria. Of those 100-200 gram-positive bacteria, 60-80% should be rods and

20-40% cocci. Our results indicate that apart from detection of gram-negative bacteria, which is a clear pathogen, faecal checks to ascertain bacterial abundance and balance of flora is subject to too many variables. It has been a paradox for results to indicate a bacterial imbalance in birds assumed to be healthy based on other parameters measured during the routine health checks, while indicating a perfect balance in birds that had other clear indications of disease at the time of sampling

Collecting faecal samples is a time-consuming process as they have to be collected fresh, uncontaminated and of known origin. The procedure involves the person responsible for collecting the samples spreading clean newspaper under the bird's favored perch and then waiting for it to defecate. The large investment of time involved in faecal collection from the large number of Spix's Macaws at AWWP, in combination with the conflicting conclusions from fecal analysis, inevitably prompted a thorough questioning of the factors affecting the bacterial floral abundance and balance and therefore its inherent value as an indicator of health. Consequently, AWWP decided to undertake a short-term, intensive research project to learn more about the bacteriology of faecal samples. One of our findings indicates that it is best to take faecal samples very early in the morning before birds are fed in order to have enough bacteria present to be statistically useful.

The Al Wabra Breeding Facility

Our two primary Spix's Macaw breeding facilities have been constructed not only with breeding in mind but also the well-being, health and bio-security of the birds. Each of the 20 isolated aviaries has not more than one pair in each. Each aviary has an indoor and outdoor section, which are accessible to each other via two windows big enough for the birds to fly directly through. The indoor sections measure 4mX4mX2.8m and have a service corridor at the front, which is where the aviaries are accessed and where the food and water station is accessed without the need to go in with the birds. The corridor continues at a right angle to flank one side of the inside section and finishes with an access door to the outside compartment. The side corridor is where inspection hatches for nesting boxes can be accessed as well the door to go inside with the birds and a transparent fronted compartment for housing surveillance cameras.



FS – Feeding Station, NB – Nest Box, AD – Access Door, AC – Air Conditioning

Each inside section has its own air-conditioning unit fitted to the front corridor wall but facing the direction of the side corridor to prevent cooled air blowing directly at the inside pen. Lighting is controlled by automatic timers and adjusted according to the length of the day. Glass windows are also installed above mesh roof level of the inside compartment so as to allow more daylight inside. The substrate is a 5cm layer of fine dune sand and below that concrete. Every second day the sand is sieved clean of any rubbish and faeces and each aviary is equipped with its own tools for cleaning and foot wear to avoid the spread of unwanted pathogens between aviaries.

The outside sections are 5.6mX5.6mX2.8m. A 25cm distance separates two layers of mesh roofing, in between which lies either a manually or automatically controlled rain system. Because of the extreme heat in Qatar during summer, the top layer of roofing is completely covered with shade netting. The shade netting also doubles as protection against droppings from wild birds landing where the Spix's Macaws might access them.

The first ten of these aviaries constructed have concrete flooring with potted plants inside for beautification and enrichment for the birds. The other ten aviaries constructed have natural substrate with the top 6-8inches consisting of washed sand typically used in mixing concrete for construction. The washed sand has the advantage that it is mined from quarries and therefore not exposed to wild birds or pollution. All the clay and most of the salt are flushed away during the washing process leaving a product that is quite coarse, clean and drains very well. These aviaries are landscaped with a variety of grasses, shrubs and small trees, but in a way that does not impede flight space. Several of the plant species will provide seeds and fruits that can be eaten by the birds. Solid concrete walls divide each pen, thereby denying visual or physical contact between birds in adjacent aviaries.

Diet Management

We have found that strict dietary provisioning is a very important consideration in the management of Spix's Macaws. Unlike certain other macaws, they should not be allowed a high fat content in their diet. When given nuts and seeds at liberty, they will eat a lot more than necessary, especially sunflower seeds which appear to be the worst culprit for excessive weight gain in this species. To exemplify my point, we had one Spix's Macaw arrive in Qatar from Switzerland that weighed 546g and was so obese he could not fly. In comparison, the average weight of AWWP's 20 male Spix's Macaw is 318g (AWWP, unpublished data). After being placed on a strict maintenance diet, this bird eventually lost the excess weight and is now a healthy 346g and can fly perfectly.

We have worked very hard and consulted many experienced people to develop the current maintenance and breeding diets for Spix's Macaws (see table 2). It was important for us to not only develop a diet that was as balanced as possible but also one which the birds willingly accepted and which simultaneously aided enrichment. To further enrich feeding time, the birds are made to work for their afternoon dry food mix by mixing it in a shallow tray with clean wood shavings. So instead of spending only 5-10 minutes to finish their dry food, they have to spend at least a half hour and are more likely to return to the food tray throughout the remainder of the day to search for more food.

We are confident that the current diets are beneficial for the species as we see improvements in the overall health of the population with every round of annual health checks we perform. Anecdotally, we can comment that the feather condition of the birds has improved and they are much more active than in the past. At the time of writing we had not lost a Spix's Macaw since 2005 and the incidences of illness have been negligible during this time. In 2007 we have documented an increase in the egg size and chick hatch weights compared with those from 2005 and 2006, all strong supporting evidence for the successful formulation of our current diet.

Table 2.1: Spix's Macaw Diet at AWWP

Food Items	Spix's Macaw Maintenance Diet		Spix's Macaw Breeding Diet	
	Morning: per pair	**Afternoon: per pair**	**Morning: per pair**	**Afternoon: per pair**
Parrot soft food mix:	2 table spoon (30g).	-	3 table spoon (45g).	-
Fruit salad:	2 table spoon (30g).	-	2 table spoon (30g).	-
Zeigler pellets maintenance ®:	4 pieces (5g).	8 pieces (10g)	-	-
Zeigler pellets breeder ®:	-	-	4 pieces (5g).	12 pieces (10g).
Milk thistle seed:	8 seeds (1g).	8 seeds (1g).	8 seeds (1g).	8 seeds (1g).
Spix's Macaw dry food mix:	-	1 table spoon (15g).	-	1 table spoon (15g).
Almonds: Mon/ Wed/Fri/Sun	-	2-pieces.	2-pieces.	2 pieces.
Walnuts: Tues/ Thur/Sat	-	2-halves.	-	2 halves.
Hard boiled egg:	-	-	1 table spoon (10g).	-
Crushed mineral block:	-	(1g).	-	(1g).

Table 2.2: Descriptions of food mixtures

Parrot soft food mix	
• Mixed frozen veg:	5 parts.
• Boiled beans and pulses:	3 parts.
• Sprouted seed:	2 parts.
• 100% cranberry concentrate:	2ml/per cup.
Lear's macaw dry food mix	
• *Harrison maintenance pellets (fine)®:*	5 parts.
• *NutriBird - P15 pellets: 50:50 original/tropical®:*	2 parts.
• *Prestige - Parrot premium mix®:*	2 parts.
• *Prestige - Tropical finch mix®:*	1 part.
Fruit Salad	
• Apple	• Mango
• Banana	• Paw Paw
• Broccoli	• Pear
• Red Bell-peppers	• Orange
• Celery heads	• Carrot
• Endives	• Red Chili

Data Collection and Record Keeping

Data collection and records are an important and major part of managing AWWP's large population of Spix's Macaws. AWWP has its own digital record-keeping program which we refer to as our "Stocklist Program". It is similar to the ARKS record keeping system used by zoos but is more advanced, user friendly and specific to our record keeping needs. All animals acquired or bred at AWWP since 2000 are assigned a stocklist number. The AWWP Stocklist Program is designed to hold a complete history of an animal, from the day it was born/hatched/accessed to the day it dies, identification, parentage, morpho-metrics, transfer history, breeding history, behavioral observations, medical history etc. The section for attachments provides a very useful function, where we can attach, for example, endoscopy photos or incubation and hand-rearing data.

To compliment the digital record we also keep hard copy records for every animal, which we refer to as an Individual Records (IR). Every IR has a medical chart where details of any illness symptoms, clinical findings or subsequent treatment are entered before being encoded later into the Stocklist Program. If the Bird Department has a health concern for one of their birds, part of the process of informing the Veterinary Department is to present them with the animal IR at the same time so that the veterinarian can immediately view the history of the animal. The IR is only returned once the case is closed and after the written data is entered into the Veterinary Departments own digital records. The IR is also where all the paperwork related to an individual specimen is kept, for example; DNA sexing certificates and laboratory reports.

AWWP's Blue Macaw Coordinator also has the responsibility of managing the international studbook for the species, using the ISIS studbook management program known as SPARKS. When taking over the studbook in 2006, it required much updating, amending and data-entry. In some cases, data was scarce or contradictory, and consequently it was essential to review historical records. Once a year a studbook report

is prepared which contains statistical analysis of the population, an historical overview and recommendations for the following year. When finished, this is then sent out to all the holders for comment before being given the seal of approval from IBAMA as an obligatory document.

Reproductive Success and Genetic Considerations

It is well established that the Spix's Macaw population is highly inbred (Presti *et al.,* in preparation) and that inbreeding presents serious challenges when it comes to managing this species. Infertility, embryonic mortality and physical deformities are common features of the breeding program which substantially impact recruitment, consequently hindering the recovery effort. The reproductive statistics for AWWP's Spix's Macaws are a cause for great concern—from 121 eggs laid; only 15 chicks have been recruited into the flock. Table 3.1 highlights the assumed impact of inbreeding on Al Wabra's breeding effort between 2003 and May 2007.

Table 3: Reproductive statistics of AWWP flock 2003 – May 2007.

Number of egg-laying females	10
Number of eggs laid	121
Number of eggs broken	5
Number of eggs viable	116
Percentage fertility	28.4% (n = 33 from 116)
Percentage early embryonic deaths	12.1% (n = 4 from 33)
Percentage mid embryonic deaths	18.2% (n = 6 from 33)
Percentage late embryonic deaths	24.2% (n = 8 from 33)
Percentage embryonic deformities	18.2% (n = 6 from 33)
Percentage hatchability	45.5% (n = 15 from 33)
Percentage chick survivability	100% (n = 15 from 15)
Number of chicks reared to fledge	15

The good news is that Dr. Cristina Miyaki and her colleagues from the University of Sao Paulo have launched a Spix's Macaw DNA analysis project to map the gene pool of the individuals participating in the IBAMA managed breeding program by analyzing the micro-satellite markers unique to each bird. On the basis of this data, it is possible to assess the genetic compatibility of every possible pairing combination. Each hypothetical pairing is ranked from "A" to "E", with "A" pairings being the most genetically compatible. The technology is so accurate that it would even be able to indicate best genetic pairings between siblings, if ever other breeding options become desperately scarce. Blood samples have been collected from

all of AWWP's Spix's Macaws. So far we have results from 23 birds, and the remaining analysis should be completed sometime in mid-2007. The key to future breeding success is compatible pairings, and this does not just mean two birds that are impressed enough with each other to reproduce. There are four factors that are taken into account when selecting mates for Spix's Macaws:

1) Each individual Spix's Macaw has a reproductive ranking based on a scoring system for gonads when viewed via endoscopy during a health check, combined with the individual's breeding history, if it has one. Pair selections begin by identifying the most compatible female for the highest ranked single male until all the males have a partner. We do it this way because we have more females than males. A female is selected based on the remaining 3 factors.
2) Genetic compatibility.
3) Health status of the potential pairings is taken into account to prevent any combinations that present a high risk of disease transfer. Every Spix's Macaw at AWWP has a health status score which is a combination of a color and a number, as defined in table 4. Based on this scoring system, the most desired outcome is to pair birds which are in the same color group, however pairing green with yellow or yellow with red is acceptable under certain circumstances but never do we pair a red color bird with a green one.
4) Age of birds in potential pairing. AWWP likes to pair birds that are of a similar age so that we can maximize the breeding life span of the pair.

Table 4: Health status scoring system

Group Virus Risk Assessment: based on the results of viral tests and biopsies.

- **Green:** No risk, all pairing possible
- **Yellow:** Less risk, restricted pairing
- **Red:** High risk, virus carrier, restricted pairing, never with green color

Individual Health Assessment: based on health check (X-ray, blood screens, clinical signs etc…)

1. **Very good:** If no abnormalities are detected in all tests and examinations
2. **Good:** If it is positive for one test but no clinical signs
3. **Fair/average:** If it is positive for two tests but no clinical signs
4. **Below average:** If it is positive for two tests and clinical signs
5. **Poor/bad:** Most of the tests positive and clinical signs

If the pairings work and breeding occurs, we aim to maximize reproductive output but not to the extent that short term success can compromise breeding consistency or longevity. We manage our pairs for a maximum of two clutches of eggs during the spring breeding season; the typical clutch size is four eggs. Often at AWWP we have a second breeding season following the summer and, if pairs which laid in spring choose to lay again in the fall, then we do not prevent them nor do we encourage them to do so.

Controlling disease transmission, in particular PDD, means that several very useful breeding management strategies have not been used to improve breeding results. Many of the current pairings are forced pairings because we have not been able to flock birds for self-selection because of our strategy to control PDD. Unlike most parrots where compatibility is easily assessed, it seems that with the Spix's Macaw almost any two birds can be put together, whether a pair or same sex,

and whilst giving the impression that they are a perfectly bonded pair; in reality this is often not the case. Another potentially beneficial strategy is off-season flocking of breeding and bonded pairs and now that the health situation is improving we can start to seriously consider this option.

To date, we have not allowed any parent rearing since there is less risk involved with hand-rearing. Given the difficulties associated with breeding Spix's Macaws, our position has been to not risk parent rearing yet. The other reason for hand-rearing has been has been to eliminate disease transfer but this has not proved completely effective as we have likely cases of vertical transmission of APMV, AHV, APV and Psittacine Beak and Feather Disease (PBFD) viruses. In the cases of APV and PBFD, these viruses have been detected during histopathology of dead Blue-headed Macaw (*Primolius couloni*) embryos but not in Spix's Macaws. APMV as well as AHV, which was discussed earlier, have been detected in juvenile Spix's Macaws that have been hand-reared from the egg and had no contact with other birds. The mother of the offspring to test positive for APMV titer was also positive for APMV titer.

Incubation and Hand-Rearing

For reasons unknown Spix's Macaw eggs are difficult to artificially incubate, and the problem lies in inadequate development of the blood vessel network. Fortunately, however, Spix's Macaw pairs are generally very good incubators so the need to cross-foster eggs or artificially incubate is rare. The eggs are left with the parents until they start to draw down into the airspace and always removed before the external pip. The egg is closely monitored during the hatching process, with the details recorded in an incubation data sheet along with details such as lay date, egg morphometrics - including weight loss, embryo development, parentage and an individual egg-code (dams Stocklist No./egg number from that female for that year/and the year, Example 4269-1-07). The hatching process is also recorded onto a hatch report form although the primary focus of the hatch report is for assessment of the chick after hatching. Details are recorded such as hatch weight, Stocklist Number, yolk sac retention, yolk sac absorption, responsiveness, skin color and any physical abnormalities.

Immediately after hatching, we weigh the chicks and apply betadine wound cream to the abdomen. The chicks are then given 2-3 hours to settle and dry after hatching before being given their first drink of fluids (equal parts Lactated Ringers Solution/Glucose/Saline) and PT12® (Re-Scha Company), a commercially available strain of avian lactobacillus. Fluid continues to be given every two hours until the remainder of the yolk sac has been absorbed before starting on hand-rearing formula. PT12 continues to be given once a day for the next 14 days. Every time a chick is fed, the following data is recorded: date, time, feed number, temperature, humidity, weight before and after feeding, total volume fed, percentage of solids to liquids and a section for comments which may include observations such as the crop status, feeding response, eyes opening, pin feathers emerging etc. Cloacal and choanal swabs are taken for bacteriology testing on days 3 and 7 and every seven days thereafter until two weeks after the bird has weaned. Closed iodized aluminum leg bands (Size#12 – 9.53mm or 3/8") are fitted when chicks are approximately 120 grams or 15-16 days old, which is around the same time they start opening their eyes. Each Spix's Macaw bred at AWWP has a unique leg band color combination and ring code.

Like with the adult Spix's Macaws we have found that diet is also very important when hand-rearing chicks as they are prone to gain too much weight if fed too rich a formula or fed too much or too often. Hand-rearing formulas designed for large macaws are not suitable for Spix's Macaws, standard low fat formulas are much better. One of the side effects of over feeding Spix's Macaws is regurgitation of formula in the seconds and minutes following feeding. In 2006 we had a biology student (Heidi Groffen MSc.) undertake an exhaustive Masters Research project on the subject of regurgitation in hand-reared parrots. The data clearly showed a link between over feeding and increased incidences and amounts of regurgitation in cases where no pathological cause, such as yeast infection could be determined. When a healthy chick regurgitates after feeding, we respond now by immediately reducing the frequency of feeds or the amount of food being fed and this has proved effective at stopping the problem.

To prevent the spread of disease, we have a policy whereby persons hand-rearing Spix's Macaws cannot go from enclosures housing adult birds to the nursery without first changing clothes and washing hair. Our main concern is bringing APV into the nursery as it is an extremely tough virus and easily transmitted. To date our policy has been successful as none of the offspring reared at AWWP have suffered from a clinical APV infection and none have tested positive for an APV titer. We have a similar policy to manage the adult population which is based on the health status of individuals or pairs. All feeding and cleaning is done in the order starting from lowest risk birds to highest risk birds and there is no going back to an aviary with low risk birds after being with high risk birds without changing clothes or donning a lab coat first.

Further Problems and Solutions

Given the large number of Spix's Macaws at AWWP, breeding success has been very limited although we anticipate that in the coming years breeding results will improve. Breeding facilities are complete and, as a result of years of disease screening, we have a much clearer picture of the health status of the individual birds and the population as a whole. The health problems we took on when we acquired the Spix's Macaws are the primary reason why the reproductive rate has been low. Another major problem affecting breeding results is the poor reproductive condition of many of the birds that should be old enough for breeding but have immature testes or ova. We are currently exploring the possibility of conditioning as many as 10 Spix's Macaws for voluntary hormone therapy via a nasal spray in order to try and stimulate the maturing of their gonad(s). An interesting trait of the Spix's Macaw at AWWP is very late maturation, which is something we have determined via endoscopy. The youngest Spix's Macaws to breed from this bloodline here at AWWP and at BII have been 8 years old and the youngest to actually produce fertile eggs have been 10 years old. Similarly, the average age of pairs producing fertile eggs for the first time is approximately 12 years. We have endoscoped some 8 year old females, which have been nest active or been observed mating, that have had ovarian development similar to that expected at a typical six month old parrot of similar size.

It is hard to determine whether the slow maturing in these Spix's Macaws is genetics related, health related, environmental or some other factor we have not considered. It is unlikely to be species specific as there are historical records of Spix's Macaws breeding much earlier. Until recently we strongly suspected that it was a genetic characteristic of

our bloodline but, after a recent case, we have been forced to seriously consider the other factors. A 20-month-old female was endoscoped in January 2007, primarily to determine her ovarian development in comparison to those of her female siblings that were bred at BII in the Philippines in 98', 99' and 00'. To our delight and surprise, this young female's ovary was significantly more mature than those of many of her much older siblings. Based on her behavior and ovary maturity she should be capable of breeding in 2008.

It is possible that substantial differences in facilities and environmental conditions between AWWP and BII could be responsible for substantial differences in neuro-endocrine development in young birds, particularly the pituitary gland responsible for controlling sexual maturity. The BII facility is a commercial breeding operation and does not provide much in the way of flight space or environmental enrichment for its birds. On the other hand, AWWP is non-commercial and our animals are kept in large aviaries and provided with environmental enrichment. In the case of Spix's Macaws, we flock the young birds in large (15-21meters) planted flights with natural branches, substrates and rain system to keep them enriched. The effect of environmental enrichment on brain development and sexual maturity is an area of avicultural science that probably warrants more attention as the Spix's Macaw is not the only species where breeding from first and second generation birds has become a long waiting game.

Conclusion

Managing Spix's Macaws at AWWP has been a challenging, sometimes frustrating but ultimately rewarding experience for the dedicated people involved. It is hoped that the effort and funds we have invested will ultimately lead to the species reestablishment in nature, in pursuit of the goals of Sheikh Saoud and the AWWP staff. We also hope that the knowledge gained at AWWP from our experience with Spix's Macaws will be beneficial to all facets of aviculture but especially to captive breeding for conservation.

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Explanation of proprietary nutritional products mentioned in text

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