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ACTION PLAN FOR THE CONSERVATION OF THE SPIX'S MACAW - *Cyanopsitta spixii* CAPTIVE PROGRAM PROTOCOL - VERSION 2018

Justification

Spix's macaw is currently one of the most threatened birds in the world. It is probably extinct in the wild and less than 200 individuals remain in captivity in the world. The last wild individual was registered in 1990 and, despite the efforts towards its conservation, there has not been any confirmed sightings since 2000. The recovery of the Spix's macaw relies on the success of the reintroduction of captive-bred birds into a suitable site within its historical range. The *ex situ* conservation, namely the Captive Program of Spix's Macaw, was approved by the ICMBio, through the Ordinance ICMBio n°. 212/2013. The Ordinance ICMBio n°. 248/2016 approved the working group of Spix's macaw Captive Program.

Until recently, the poor rate of reproductive success was of concern to the Spix's macaw Captive Program. Breeding output was not at a desirable level, even though egg production was high. Microscopic research on non-developing eggs conducted by Al Wabra Wildlife Preservation (AWWP) in collaboration with the University of Sheffield (UK), revealed that approximately 50% of the eggs laid at AWWP during the 2010 season were actually fertile, but only around 10% (6 of 51) of the total number of eggs laid during that season were viable. The research also indicated that the males within the population have an overall low sperm count. These results, along with other supporting data indicated that there is most likely a genetic and hormonal problem adversely affecting reproduction. In 2012, Daniel Neumann and Dominik Fischer, from Giessen University, collected semen from the males Spix's macaw kept at AWWP to quantitatively and qualitatively analyze their production and test an artificial insemination technique. The results showed that these birds had very little semen, with vastly fewer spermatozoa compared to other parrots of similar size. In 2013 AWWP signed a three-year contract with Parrots Reproduction Consulting (Daniel Neumann and Heiner Müller) and started to inseminate Spix's macaws. In the 2013 breeding season two chicks were produced using artificial insemination. The results were obtained again in 2014. Using hormone analysis, dietary research, egg analysis, genome sequencing, genetic similarity estimates, behavioral analysis, as well as semen collection and artificial insemination, AWWP have been able to fine tune the science needed to help this population grow. Also in 2014, all holders in Captive Program (AWWP, Association for the Conservation of the Threatened Parrots - ACTP and Nest) produced chicks in the same year for the first time and the first two chicks were hatched by natural incubation in Brazil. In 2015 AWWP produced a record 16 chicks in one season, and this record was broken again in 2016 with 18 chicks produced. Most importantly, there are chicks produced from artificial insemination with genetics from birds that had never produced offspring before. In 2015, AWWP and ACTP produced a total of 20 chicks; in 2016, 23 chicks; and in 2017, 26 chicks.

Another challenge was female sex bias in the Spix's captive population, which is often the case with small genetically strained populations. However, over the last years (2012-2017) offspring was produced at a 50:50 ratio (32 males and 32 females) compared to a 35:65 ratio (6 males and 17 females) in the previous 6 years. This is a remarkable turn of events and this 50:50 sex ratio in the young birds combined with the larger numbers been produced should improve the population pyramid greatly over the coming years. In addition, the Cornell Medical College in Qatar's Genomics core lab has produced the full genome sequence of every individual in the population, which will help with the pairings and thus increase natural breeding, as well as furthering research in the direction of reproductive research for the species.

Most of the problems faced in the first years of Captive Program hold by ICMBio and holders (2012-2017) have been addressed and in most cases solved in the last 5 years. We made the required transfers to

establish the pairs recommended by the studbook keeper, used the technology to produce many chicks, and most of the health issues (*e.g.* bornavirus, paramyxovirus, circovirus and polyomavirus) have been eliminated. Currently the population has a great number of fertile young birds. Some of the old, infertile or physically compromised birds are not included in the breeding population and are available to the disclosure program. Young chicks not involved in the breeding are also available for other means of funding and program progression, in example of two young birds sent by ACTP to Jurong Bird Park in 2017.

Finally, the captive population of Spix's macaws is stable, with an increasing population. More than breaking records, these results show that Spix's macaws can be reintroduced soon, however the efforts have to be continuous. Then we present the planning for the Captive Program for the next years.

GOAL - Manage the captive population at the highest standards, utilizing the latest advancements in management and reproduction to increase the population as rapidly as possible whilst also retaining as much healthy genetic diversity as possible.			
Specific Objective 1 - Management of the captive Spix's macaw as a single population involving all the holders who agreed to accept and fulfill the protocols established.		Specific objective 2 - Have viable breeding populations, with surplus birds available for the reintroduction before 2022.	
Actions	Deadline	Responsible	Comment (if required)
1. Revise the existing protocols, update them as new scientific studies are developed and implement them on all the holders	Continuous	Camile Lugarini and Cromwell Purchase	
2. Confirm identification of the birds, verify the kinship, review the pedigree and the genetic similarity value of the total known population of the species to base the pairing up.	Continuous	Cromwell Purchase	
3. Perform the genetic analysis of all birds.	Continuous	Cristina Yumi Miyaki and holders	Prof Cristina Miyaki of Sao Paulo University can provide important genetic information for investigating the relationship between individuals and the relationship of every individual to the population. The Cornel Medical University in Qatar (Prof Joel Malek) completed the full genome sequence of all individuals in the population. This will help to more accurately ascertain the best breeding options as well as possibly find fatal genes that need to be removed from the population. Given the problems experienced due to a lack of genetic diversity within the Spix's macaw population, this information is a very powerful tool for breeding management.
4. Establish banks of viable samples for DNA extraction (tissue or blood samples), of live cells and of sperm from all the birds in the captive, aiming for long-term in-vitro conservation and joining the Frozen Ark and Genome 10K projects.	Continuous	Cristina Yumi Miyaki	Only samples for DNA extraction are available. There is only one cell culture in San Diego Zoo.
5. Perform health management, by means of standard exams of all the Spix's macaws.	Continuous	Holders	
6. Breed as many birds as possible.	Continuous	Holders	Hopefully improved pairing strategies and maturing of sub-adult birds will lead to improved breeding results.
7. Prioritize breeding efforts to ensure that birds with high genetic importance receive the best possible opportunity to	Continuous	Coordinator, studbook keeper, holders	Genetically important birds should be provided with a choice of mates to increase the chances of pair harmony.

reproduce.			
8. Conduct bird pairings as indicated by the studbook keeper.	Continuous	Cromwell Purchase and Camile Lugarini	
9. Perform artificial insemination.	Continuous	Cromwell Purchase and Daniel Neumann	Hugely successful tool in the Spix's program, allowing us to breed with excess females as well as mix genetics and use semen from males that have never reproduced naturally.
10. Provide specimen updates to enable studbook and coordinator to remain current and annual report to be produced.	Continuous	Holders	Successful hatching of chicks should be reported as soon as possible to enable the Studbook to remain current at all times. Any other relevant data requested by the studbook manager or coordinator, such as lay dates and fertility status, should be submitted promptly.
11. Incorporate more birds from private European collections into the breeding program.		Ugo Vercillo, Martin Guth, Cromwell Purchase	The more birds incorporated into the studbook managed breeding program the better chance we have to produce more offspring.
12. Flock chicks with Illiger macaws.	2018	Cromwell Purchase	To start this flocking at the end of 2017, in the meantime all young Spix's will flock together in groups.
13. Carry out a Population Viability Analysis of the Spix's macaw and validate in workshop.	2019	Camile Lugarini and Cromwell Purchase	
14. Produce surplus birds for reintroduction.	2020	Cromwell Purchase	Viable population. Excesses being produced from 2017.
15. Concordia farm in Brazil needs to have facilities setup on it for breeding, release and management.	2018	Martin Guth, Cromwell Purchase and Ugo Vercillo	ACTP with the help of the Spix's Advisory Group will establish a breeding, management and release center in Brazil in 2018 and target to have facilities ready to house Spix's Macaws in late 2019.
16. Transfer 50 Spix's Macaw to Brazil	2019	Martin Guth, Cromwell Purchase and Camile Lugarini	
17. Disclosure program and funding with old, infertile, physically compromised birds or young chicks not involved in the breeding.	Continuous	Cromwell Purchase and holders	In 2017 was sent the first young birds for a zoo for funding of <i>in situ</i> conservation.



SPIX'S MACAW CAPTIVE PROGRAM PROTOCOLS

1. HUSBANDRY

The following guidelines serve as minimum standard requirements for keeping Spix's macaws in captivity as part of the official captive breeding program:

- ❖ For short-term housing (<1 year) an aviary must provide a minimum flight length of 5m and must be at least 2m wide x 2.4m high.
- ❖ For long-term housing an aviary must provide a minimum flight length of 9m and must be at least 2m wide x 2.4m high. Ideally Spix's macaws that are not part of the breeding program (such as young birds) should be housed in large flocking aviaries with a flight length of 10-15m x 5m wide x 4m high.
- ❖ Aviary mesh should be a minimum of 1.5mm thick, a maximum space dimension of 65mm² (25mm x 25mm). Smaller mesh dimension is preferable, as this reduces accessibility for parasites. Aviary mesh must not pose a significant threat of heavy metal poisoning. All wired mesh should be welded prior to galvanization.
- ❖ Aviary must provide adequate protection from wind, rain and sun. A minimum of one quarter of the aviary should have a solid roof. The aviary should provide shelter. Measures should be taken to protect Spix's macaws against weather extremes: heating should be provided if temperatures fall below 10°C; cooling in the form of air-conditioning and/or misting/fogging should be provided if temperatures go above 40°C.
- ❖ If Spix's macaws are housed in areas where extreme weather events such as sandstorms, hurricanes or blizzards occur, the aviary should have a protected indoor area where the birds can be secured.
- ❖ Aviary must have a safety corridor to prevent birds escaping whilst entering and exiting. Every Spix's aviary should be properly protected from escapes, access by passerines, other birds and pests.
- ❖ Aviary floor must be of a substrate that drains quickly in order to prevent birds from drinking from puddles on the ground.
- ❖ Aviary must be fitted with a variety of perching options of different sizes, textures and locations to provide access to sun and shelter.
- ❖ Non-toxic live plants should be added to the aviary. If planting is not an option, potted plants will do, or if the aviary is suspended, then plants around the aviary will suffice. If plants are planted around the aviary but allowed to grow into the aviary it should also suffice.
- ❖ Ideally all Spix's macaws should be kept isolated from contact with other birds. There should be a separated fully equipped section for Spix's macaws with a closed physical barrier (e.g. wall) or a minimum distance of 10m between Spix's and other parrots. When Spix's macaws are kept in close proximity to other birds, the other birds must be tested for selected pathogens (item 4.6.).
- ❖ In case the Spix's macaws cannot be in an isolated facility, all the captive birds within 100m must be tested. If a facility is keeping other bird species within 200m, the list of species will be provided to the coordinator in order to evaluate the need to run tests. This will be done on a case by case evaluation, accordingly to bird species (e.g. Psittacines or not) and relevant documentation to show the health status of the collection. Costs are covered by the holder. The working group of the program could visit the site and give directions regarding which diseases must be tested and how to proceed. In case other Psittacine birds have to be introduced in a flock of Spix's macaws, either for experimental trials, to be

used as trainers for younger birds, or to stimulate mating behavior, they will be tested and treated with the same sanitary protocols as if they were Spix's macaws.

- ❖ New birds and every Spix's macaw should be fully tested prior to any transportation (item 4.6). Testing should be performed during the 30 days quarantine period by both holders (90 days for Avian bornavirus), the recipient and the dispatcher. The institution sending the Spix's macaw should always provide to the receiving holder all documentation and full clinical history of the bird to be transferred including examinations performed on the Spix's macaw before the transport takes place.
- ❖ Work clothes must be cleaned every day. Ideally, there should be exclusive keepers for Spix's macaws. It is also important that keepers who directly manage Spix's Macaws cannot have personal pet birds in their homes, due to the possible pathogen transfer.
- ❖ Efforts should be made to minimize physical interactions with Spix's macaws during their daily routine (birds on shoulder, head, etc...) by staff responsible for Spix's macaws and also visitors.
- ❖ Visitors can be allowed at the discretion of the holder when necessary and should be monitored during their visit to the facilities. In those cases, visitors must follow the biosecurity measures established by the bird holder. Visitors cannot be in contact with other birds 72 hours before.
- ❖ A recommended minimum of two nesting options should be provided to all Spix's macaw pairs set up for breeding. Slanted nest boxes are preferred by the animals in one holder. It is suggested that all nest boxes be constructed and fitted with surveillance cameras to monitor nesting activity. Outside access to the nest box must be present and a safety corridor or other structure that prevents escapes through the nests has to be included.
- ❖ A surveillance camera monitoring the aviary should be placed in order to observe behavioral interactions and to monitor the health of birds without the added stress of human presence.
- ❖ Catching nets and transport boxes should be cleaned and sterilized before being used to capture and contain Spix's macaws.
- ❖ Each facility should have nets and transport boxes exclusively reserved for Spix's macaws. Each aviary/cage must have its own equipment and material exclusively reserved for Spix's macaws use.
- ❖ Any physical activity (catching) involving staff and Spix's macaws should be conducted in the preferred presence of a veterinarian. All incidents must be photographed and senior staff called immediately to verify any incident (this is for the protection of both the birds and the staff) or, preferably, surveillance camera(s) should monitor the entire aviary.
- ❖ The entire routine, changes in behavior, food, staff, etc... must be documented.
- ❖ Spix's macaws should be fed before all other birds on a one way work flow, and anyone that has had direct contact with other birds should not return to Spix's macaw aviaries without showering and changing clothes.
- ❖ Spix's macaws must be provided with clean, fresh drinking water at least twice a day in a clean and smooth surfaced dish (e.g. stainless steel or smooth glazed terracotta). Birds in rehabilitation for release may not apply as they should have options that they will find in the wild. Care should be taken to position their water in a location to prevent it from being soiled by feces or food waste.
- ❖ Spix's macaws need to be provided with food preferably twice per day in clean, dry dishes, ideally in different forms according to the environmental enrichment program for each group. Any perishable food items should be removed before they have a chance to spoil, including waste that has landed outside the food dish.
- ❖ An environmental enrichment program should be in place in all holding facilities. The program must be planned according to the purpose of the captive facility (breeding, flocking, rehabilitation for reintroduction, among others).

- ❖ All holders should provide a secure facility. Preferably, there must be permanent presence of people on-site at the property holding Spix's macaws. Security measures are up to the discretion of the holder, and could include lighting, and/or day/night surveillance cameras installed at entry points to Spix's macaw aviaries.

2. DIET

Dietary choices are left to the discretion of the holder, however, care should be taken to ensure the birds receive a balanced diet based on the best information available and holders should be mindful that Spix's macaws, especially the older ones, are prone to obesity. Regarding the best information available, this protocol should include in the future a full bromatological analysis of the ideal Spix's macaw diet, as well as a bromatological analysis of items known to be ingested in the wild in the past. Therefore, food items which are high in energy should only be fed in small quantities and never ad-libitum. Behavioral enrichment of Spix's through the way food is offered should be considered, and not only the biological properties of that food.

In Tables 1 to 4 are diet recommendations from AWWP that can be used as a guide if other holders prefer.

Table 1 – Maintenance Diet (AWWP).

Morning: Per pair	
Parrot soft food mix	2 table spoons (30g)
(during Winter)	3 table spoons (45g)
Fruit/Veg salad	2 table spoons (30g)
Milk thistle seed	8 seeds (1g)
Almond or Sunflower	(2g)
Afternoon: Per pair	
Harrison's Adult Lifetime Fine	1 table spoon (15g)
(during Winter)	2 2/3table spoons (20g)
Milk thistle seed	8 seeds (1g)

Special Instructions

Morning: Add one quarter of walnut per bird in the woodchip tray when the morning food dish is removed.

Afternoon: Mix Cranberry Concentrate with Harrison's pellets (1ml per 20g, e.g. 25ml to 500g). Then add Harrison's Avix Booster (1ml/100g, e.g. 5ml to 500g).

Table 2 – Breeding Diet (AWWP).

Morning: Per pair	
Parrot soft food mix (see Table 3):	2 table spoons (30g)
(during Winter)	3 table spoons (45g)
Fruit/Veg salad (see Table 3)	2 table spoons (30g)
Milk thistle seed	8 seeds (1g)
Almond or Sunflower	(2g)
Afternoon: Per pair	
Harrison's Adult Lifetime Fine	1 table spoon (15g)
(during Winter)	2 2/3table spoons (20g)
Milk thistle seed	8 seeds (1g)

Special Instructions

Morning: Add one quarter of walnut per bird in the woodchip tray when the morning food dish is removed.

Afternoon: Mix Cranberry Concentrate with Harrison's pellets (1ml per 20g, e.g. 25ml to 500g). Then add Harrison's Avix Booster (1ml/100g, e.g. 5ml to 500g).

Table 3 – Contents in detail (AWWP).

Parrot soft food mix	
Mixed frozen veg	5 parts
Boiled seed	3 parts
Sprouted seed	2 parts
Prime Vitamin & Mineral Supplement	12g per/1kg
Fruit Salad	
Apple	Mango
Banana	Paw Paw
Broccoli	Pear
Carrot	Orange
Celery heads	Red Bell-peppers (Capsicum)
Endives or Kale	Red Chili
Mixed Frozen Vegetables	
Carrot	String beans
Green peās	Sweet corn kernels
Boiled Seed Mix Ingredients	
Chick peās	1 part
Egyptian foul	1 part
Large foul	1 part
White beans	1 part
Whole corn	2 parts
Green peas	1 part
Special Dinner Mix	1 part
Yellow peas	Lentils
Green peas	Mung beans
Maple peas	Safflower
Chick peas	Buckwheat
White beans	Milo
Red beans	Dari
Speckled beans	Paddy Rice
Tic beans	Peeled oats
Corn	Wheat
Vetche	
Mix 2	1 part
Milo	Pigeon peas
Safflower	Mungbeans
Sprouted Seed Mix	
Prestige Germination Seeds for Parrots	1 part
Safflower	30%
Buckwheat	15%
Paddy Rice	12%
Wheat	7%
Oats	7%
Barley	7%
Milo	6%
Hemp seed	6%
Dari	6%
Mungbeans	4%
Prestige Cockatiel Mix	1 part
Yellow millet	32%

Red millet	10%
Peeled oats	9%
Canary seed	9%
Striped Sunflower seed	6%
Wheat	5%
Safflower	5%
White millet	4%
Rape seed	4%
Hemp seed	4%
White Sunflower seeds	3%
Linseed	3%
Paddy Rice	2.5%
Peanut kernels	1.5%
Niger seed	1%
Japanese millet	1%

Table 4 – Food suppliers

Nutrition inspired by nature Versele-Laga nv	http://www.versele-laga.com	Kapellestraat 70 B - 9800 Deinze Belgium Tel.: +32 (0)9 381 32 00 Fax.: +32 (0)9 386 85 13
Harrison's Bird Foods	info@hbf-uk.co.uk	Unit 7 Windmill Road Loughborough Leics LE11 1RA Telephone 01509 265557 Fax 01509 265777
Brazilian brands have been developed and tested for quality and have been used broadly by bird breeders in Brazil (<i>e.g.</i> Nutropica Nutrição Especializada)	http://www.nutropica.com.br/	

Sprouted Seed Preparation Guide

Morning

Step 1: Wash dry seed thoroughly with fast flowing water in a bucket.

Step 2: Drain seed through sieve & rinse in filtered water.

Step 3: Return seed to bucket and fill with 1l of water.

Step 4: Add 2ml of disinfection solution and stir thoroughly.

Step 5: Allow solution to sit for 15 minutes.

Step 6: Drain seed through sieve.

Step 7: Repeat steps 3 & 4 and allow seed to soak for 12 hours.

Afternoon

Step 8: Rinse seed thoroughly in sieve.

Step 9: Repeat steps 3, 4, 5 and 6.

Step 10: Allow seed 36 hours to sprout.

Morning

Step 11: Repeat steps 2, 3, 4, 5 and 6.

Step 12: Rinse thoroughly in fast flowing water before feeding.

Step 13: Always smell the seed before feeding; it should have a sweet smell. Discard if smell is sour or irregular.

Considering the use of disinfectants controversial, as they might alter the intestinal flora, a preferable approach would be to avoid any disinfectant, and then, after soaking overnight, pass both, beans and seeds in the microwave oven for two minutes. This sterilizes them, but they remain hard enough for an increased palatability. For those not interested in using a safe disinfectant like F10, Aqueous Iodine (10%) can be used in the soaking of seeds and rinsed off in the same way as the F10 is.

3. INCUBATION AND HAND REARING

Incubators should be setup at the first sign of breeding behavior and should run throughout the entire breeding season. Grumbach incubators are preferred, but any reliable incubator can be used. There should always be more than one incubator setup at any given time to ensure a backup is ready if there are any mechanical issues. All nursery systems (incubators and brooders) should be setup with a backup power supply and surge protector. The best method is a UPS system and an automatic backup generator if the power goes out. An automated calling system that dials the staff cellphone when there is a technical issue in the nursery is helpful.

The incubators should be setup at 37.2°C and the humidity around 40%, with the rollers turning every 2 hours (12 times per day). The even number of turns means that the egg will return to its original position after 24 hours. Every morning at the same time the egg should be weighed and turned manually 180°. If the egg is developing changes will be noticed at 5 days old. Appendix I shows the data sheets that should be filled in. The percentage weight loss of the egg needs to be closely monitored to ensure the loss is between 12 and 16%. The weight loss can be managed by controlling the humidity in the incubator. Three days prior to expected hatch date the egg should be removed from the roller, with no more turning, to allow the chick to settle and orientate for the hatch process. Once the chick has externally piped the egg should be moved to a hatching brooder set at 36.9°C and 60-65% humidity. This is where the chick will hatch and any assist hatching will be performed. Always keep a syringe of Ringers lactate or Saline in the brooder so when needed it is at the right temperature. Assist hatching is an important technique to know and learn properly, there is a fine line between patience and knowing when to go in. Only experienced staff should attempt assist hatching on Spix's macaws! A Buddy MK2 is a good tool to help with the monitoring of the chicks heart rate as well as a small candling torch.

For the rearing process, Nutribird A21 (Versele Laga) can be used as the base hand rearing formula for the Spix's macaws. Before each feed the humidity and temperature of the brooder is noted on a spreadsheet along with the time and date. The concentration of the food given as well as any components added to the mixture for the specific feed and any observations like food left in crop, feces present, alert, ate well etc have to be noted. The chick is weighed before feeding and again after feeding, the spreadsheet then calculates the amount fed and percentage of body weight.

When the chick hatches the umbilicus must be checked and some antiseptic ointment should be added to prevent infection. The chick is then left for an hour to dry before any feeding starts. The first feed is just fluids (equal parts 5% Glucose/Ringers lactate/Saline), the 2nd and 3rd feeds are the same except one has a pinch of PT12 (*Lactobacillus*) added and the other has a pinch of Harrisons Avian Enzyme added.

Once the feces is no longer yellow/lime green (yolk absorbed), A21 can be added to the diet. The percentage changes over the course of the rearing process, as does the temperature of the brooder that needs to decrease over the growth period. A formula chart with an indication of the feeding percentages followed by AWWP is on Appendix II. This is put up on the wall so that mistakes are avoided especially in the early hours of the morning. With regard to temperature, it is decreased when the chick behavior shows that it is too hot (chicks panting and lying flat with their wings open), and is increased if the chick is seen shivering.

AWWP starts with 10% A21 (1g powder/9g water). Use bottled water and in each bottle (1.5L) 500mg of Calcium Sandoz is added (effervescent Calcium). To the mix add either PT12 of Avian enzyme

alternating with every feed, and 10% human bottled baby food (preference of apple/blueberry/pear). From day 3 or 4 when the chick has been moved up to 16% in intervals (10/12/14/16%) 10% fruit and veg mix is added to the mix. Often food concentration is increased at the last feed of the night and a little Palm fruit oil or coconut oil is added to the mix to sustain the chick a little longer; giving the hand-rearer an opportunity to get an extra hour of sleep while the chick does not lose weight overnight (Cromwell Purchase feeds through the night, but obviously circumstances often dictate that most aviculturists are unable to do this).

Fruit and Veg mix: 1 punned each of Blueberries/Raspberries/Blackberries, 500g frozen mixed veg (peas/corn/carrot/beans), 200g Broccoli. Do not add any apple as it is already in the baby food, but this mix can be varied to your liking or seasonal availability. This mix is blended into a fine mix and placed in ziplock bags for freezing, wafer thin for easy breaking off of pieces when ready to add to the mix.

Increasing the formula percentage is done when you see the chick is easily digesting the food within the time allocated between feeds and is no longer gaining weight as quickly as it should. On average the 10/12/14/16 percentage increases should be done by the end of day 3-4. Increasing in stages is important to allow the chick to get used to the consistency and each chick is different, so if the chick is unable to empty its crop in the time allocated for the stage of development then just drop it back down to the previous concentration for a few more feeds.

On the first 2 days of feeding, you should try to work your way up to feeding 10% of body weight per feed, once you reach the 10% it is a good average marker for the rearing throughout. Once you have the chick at 10%, feed it what it wants to eat, which in the early stages on occasion reaches 15% of body weight per feed. Do not force the chick to eat unless the chick has a problem and has no feeding reflex, especially in the latter rearing stages when the chick should be losing weight before weaning. Start feeding when the food temperature is between 40 and 43°C, if this drops below 39°C many chicks will lose their feeding reflex and spit out the food. Food should be heated by placing the cup of food in hot water, do not microwave.

Do not rear them as pets, so try to rear the chicks in groups, and show no emotion during feeding, also this means no cuddling, and as little talking and physical contact as possible, this produces chicks that incorporate well in groups and allow for better pairing for breeding birds. This also means that the chicks are weaned in about 70 - 100 days (3 months).

4. HEALTH AND VETERINARY MANAGEMENT

The protocol focuses on **prophylactic management**, the best approach to safeguard the health of captive Spix's macaws. Its main objective is to prevent the introduction and spread of pathogens, and to diagnose, control and eliminate them before they have a negative effect on the birds. Health issues must be addressed immediately particularly since many of the birds have at least part of the time been housed in multi-species collections and exposed to large numbers of other birds and potential diseases.

4.1. Common injuries and treatment

The most common injuries that require medical treatment result from:

- ❖ self-inflicted accidents in the aviary
- ❖ minor aggressive incidents between partners of a breeding pair
- ❖ accidental ingestion of pieces of wire containing heavy metals (zinc, lead, copper). The use of stainless steel mesh is advised.

Feet, wings and head are the most affected body parts. Treatment is according to standard avian veterinary practice. Severe aggression is uncommon in Spix's macaw but can lead to trauma and even death.

4.2. Critical infectious diseases and prophylactic management

Most important infection diseases for parrots and macaws are listed in Table 5. Regrettably, recent evidence has indicated that a significant portion of the extant captive Spix's macaw population (AWWP

birds) has been exposed, to various dangerous pathogens, most notably bornavirus, paramyxovirus, circovirus and polyomavirus (Deb et al. 2007, 2008, 2009; Wyss et al. 2009; Staeheli et al. 2010). This imposes severe population management constraints and will require extremely careful implementation of veterinary protocols throughout the lifetime of these birds. Prevention is the essential guideline of this protocol.

Table 5 - Relevant infectious diseases that can affect captive psittacines.

Disease	Pathogen
Psittacine Beak and Feather Disease (PBFD)	Circovirus
Avian Polyomavirus infection (APV)	Polyomavirus
Pacheco's and Pacheco's-like Disease	α -Herpesvirus 1(PsHV-1)
Proventricular Dilatation Disease (PDD)	Avian Bornavirus, and possibly other infectious agents.
Chlamydiosis	<i>Chlamydia psittaci</i>

4.3. Routine observation of behavioral manifestations of illness

Routine assessment of health is carried out on a daily basis. All Spix's macaws should be observed at least twice a day by an experienced avian keeper. All aviaries should be checked thoroughly once a month for potential hazards, this is over and above the daily observation routine. When necessary, samples should be collected to be examined in the laboratory.

Generally, observational controls are based on the following:

- ❖ behavior
 - changes in the bird's behavior (stereotypes or other abnormal behaviors, the bird is quieter, unusually sleepy, nervous, turns away from the partner...
 - physical posture
 - tail wagging (may indicate respiratory diseases, cloacal problems, or egg retention)
 - extremities (one or both wings are slightly hanging down, the bird obviously tries not to strain one of its feet)
- ❖ plumage
 - ruffled plumage, first the neck feathers and then the entire plumage, regulates the body temperature of the animal; at the first stage of a disease, quite often the bird ruffles a few neck or head feathers (which may also just be a threatening posture);
- ❖ eyes
 - the eyes should be carefully examined, they must be clear and fully open; at the very beginning of a disease, the birds' eyes are slightly closed and lose their shine; birds which are seriously ill have their eyes completely closed.
- ❖ excreta
 - attention is given to the quantity, color and consistency (liquidness) of excreta; both greater-than-usual and smaller-than-usual quantities might indicate an illness; although the color of the feces may strongly depend on the food ingested, it must also be carefully controlled; with regard to liquidness, the technician must distinguish between an excessive excretion of urine (urates and feces tend to be separated) and diarrhea (urates and feces tend to be mixed).
- ❖ ingestion of food and water
 - the technician must assess if a bird ingests food and water in normal quantities. Changes in food intake are important to monitor.
- ❖ each bird setup for breeding, its weight should be periodically monitored (whenever caught for any reason, and voluntarily every 3 - 6 months on a feeder perch for minimal disturbance) to validate that the diet they are receiving is not too high in energy. Special hidden scales could be used to monitor the

birds' weight voluntarily. Ideally diet should be balanced and based on bromatological scientific research. It is essential therefore to monitor the birds' weight to validate these parameters.

- ❖ nasal, ocular, cloacal discharges; can indicate a disease or allergic reaction, e.g. to nest substrate or food.
- ❖ bill and toes: trimming if indicated.
- ❖ leg bands: replace if any negative effect due to wrong size or if the band inscription is unreadable (additionally, it is obligatory to insert a microchip in all animals in their left pectoral muscle).

4.4. Routine controls of food and water quality and hygiene

Low quality, biologically or chemically contaminated, or spoiled food and water possibly represent the greatest health risks that Spix's macaws face on a daily basis:

- ❖ both food and water may contain pathogens (fungi, bacteria, toxins, etc.).
- ❖ the nutritional value of food may change over time (usually lower values of vitamins and trace elements, but also changes in carbohydrates, proteins and minerals) and should be reviewed regularly.
- ❖ Preparation of food should be done hygienically to minimize the risk of bacterial and fungal infection (clean work surfaces, clean hands and cloths etc...).
- ❖ It is imperative that high quality food is offered to the Spix's macaws, as well as high quality water in the following environments:
 - kitchen: for food preparation, for filling the drinking water bowls, for washing the food and water dishes.
 - nursery module: ditto.
 - clinic.
 - place water plumbing systems for sprinklers/showers above the aviaries and use water hoses for aviary cleaning, if possible.
- ❖ Food items must be subjected to intensive controls:
 - commercial food (pellets, seeds, and others) must be checked immediately after opening a bag at the breeding center, and prior to inclusion in the macaw diets by means of sensory analysis (smell, taste, etc.) to check their freshness.
- ❖ Disinfection of plates and cages. To reduce the risk of food poisoning, no perishable food items should be kept in the aviaries for a long time. Time is dependent on season due to increased temperatures in the summer causing spoiling of the food

4.5. Efficient control of hygienic and disinfection measures

Prophylactic measures must be carried out to the highest possible standard. Nevertheless, even in the case of disinfectants there is a danger of development of pathogen resistance. Each institution should use, at least three different disinfectants, on a rotation scheme.

Disinfection of the Nursery Module before breeding season is very important and has to be taken into account by all holders. In those cases it is suggested that prophylactic measures could include taking samples for bacteriological analyses from equipment used in the Nursery.

4.6. Routine medical control and sampling for disease

Biological samples of individual birds should be collected at least once in three years when testing for infectious diseases and/or parasitological analyses. Annually, each macaw must be caught and undergo complete physical examination, collecting data on nutritional condition of the bird. If a bird is over- or underweight it may require a special diet. Overweight might contribute to infertile eggs, and therefore this aspect should be given careful attention (optimal mass: Females 250 – 300g; Males 270 – 320g).

The protocol presented here can be used as a guideline when a Spix's macaw undergoes a clinical examination.

The following samples can be taken according to veterinary opinion:

- ❖ Blood (never more than 1% of body weight)
- ❖ Choanal swabs

- ❖ Cloacal swabs
- ❖ Oral swabs
- ❖ Crop swabs
- ❖ Feces
- ❖ Feathers

Table 6 shows a list of exams to be performed.

Table 6 – Etiological agent and sampling recommended in routine inspection of Spix's Macaw. **All holders should test the entire flock every three years and retest only in case of clinical signs or transfers between holders. Control of endoparasites has to be more frequent (at least once per year).**

Etiological Agent	Sample	Diagnosis Test	Recommended Management
	Blood	CBC and biochemistry	
Haemosporidia (<i>Haemoproteus</i> , <i>Leucocytozoon</i> , <i>Plasmodium</i> , microfilarias and others)	Blood smears or blood	Wright, Giemsa, Rosenfeld and/or PCR	If the macaw has a high parasitism, do the treatment according to parasites
Ectoparasites	Ectoparasites in isopropyl or ethyl alcohol 70°	Alpha taxonomy	Treatment according to parasites
Gastrointestinal parasites (protozoa and helminthes)	Feces (one faecal per day for three consecutive days, pooling the samples and performing one analysis. Sampling on smooth disposable substrate previously disinfected)	flotation or alternative method	Treatment according to parasites. Three negatives retests post-treatment.
<i>Chlamydia psittaci</i>	Cloacal/oro-pharyngeal swab – preferential. Feces (pooling three samples of swabs or feces collected every other day).	PCR	Isolation for treatment for clinical animals. After two negative tests post-treatment (after 2 and 4 weeks) the macaw can reincorporate to the breeding program. For birds without clinical signs treat and retest, as the stress of moving between institutions/pairing/adapting to new diets/climate could be triggering events for further shedding/manifestation of clinical signs
<i>Salmonella</i> spp.	Cloacal swab Feces	Culture	Treatment if the bird has clinical signs. After two negative tests post-treatment (after 2 and 4 weeks) the macaw can reincorporate to the

			breeding program. For birds without clinical signs treat and retest, as the stress of moving between institutions/pairing/adapting to new diets/climate
<i>Mycoplasma</i> spp.	Oro-pharyngeal, cloacal and conjunctival swabs Serum	PCR Serology to <i>M. gallisepticum</i> and <i>M. sinoviae</i> (this only for legal reasons if they have to be moved: parrots do not get infected with <i>M. gallisepticum</i> and <i>M. sinoviae</i>)	Isolation for treatment for clinical animals. After two negative tests post-treatment (after 2 and 4 weeks) the macaw can reincorporate to the breeding program. For birds without clinical signs treat and retest, as the stress of moving between institutions/pairing/adapting to new diets/climate could be triggering events for further shedding/manifestation of clinical signs.
Cloacal and oral microbiota	Cloacal/oral swab in Stuart or Amies Dry sterile swabs for fungal samples	Culture	Caution on interpretation - bacterial and fungal growth may not be related to disease. For clinical animals treatment according to bacterial and fungal isolates.
<i>Mycobacterium</i> spp. (<i>Mycobacterium avium</i> , <i>M. tuberculosis</i> , <i>M. bovis</i> or <i>M. genavense</i>).	Cloacal/oral swab /fine needle from suspected lesion	Gram and Ziehl Neelsen and PCR	Isolation, treatment if the macaw has clinical signs and retest after treatment. This is a screening test only for legal reasons. In confirmed positive cases further testing must be performed to investigate if it is a disease case or if it is only an environmental pathogen.
¹ Paramyxovirus and Avian Paramyxovirus type 1 (APMV-1)	Cloacal/oro-pharyngeal swab Serum	RT-PCR Serology (Hemagglutination Inhibition for screening and ELISA for confirmation)	Obligatory Notification for APMV-1 Decision depends on sanitary agency regulation for those birds kept within this country
² Alpha-herpesvirus of Pacheco (PDV)	Feces Cloacal/oro-pharyngeal swab Serum	PCR Serology (not available in Brazil)	Isolation of the bird. Serology and PCR have to be performed in all contact birds. Pairing recommendations should include only PDV negative or

			PDV positive birds with each other. PDV is an herpesvirus and survivor birds cannot be declared “safe”. Eggs from infected and survived hens should be incubated artificially.
² Polyomavirus (APPV)	Feathers/oro-pharyngeal and cloacal swab Serum	PCR Serology (not available in Brazil)	Isolation of the bird and retest in 90 days. After three negative PCR tests within 3 months, birds can be transferred or reincorporate to the breeding program. Serology and PCR have to be performed in all contact birds. AI or pairing recommendations should include only APPV negative or APPV positive birds with each other (consider artificial incubation).
² Circovirus (PBFD)	Feathers and blood	PCR	Isolation of the bird and retest in 90 days. After three negative PCR tests within 3 months, birds can be transferred or reincorporate to the breeding program. Serology and PCR have to be performed in all contact birds. AI or pairing recommendations should include only APPV negative or APPV positive birds with each other (consider artificial incubation).
² Influenza A	Cloacal/oro-pharyngeal swab Soro	RT-PCR Serology (Hemagglutination Inhibition for screening and ELISA for confirmation)	Obligatory Notification Decision depends on sanitary agency regulation for those birds kept within this country.
² Avian Bornavirus (ABV)	Cloacal swab (screening - pooling three swabs samples collected every other day)	PCR and Serology - ELISA preferential (not available in Brazil)	Isolation of the bird. Serology and PCR have to be performed in all contact birds. After three negative tests within 3 months, birds can reincorporate to the breeding program.

Aviadenovirus	Cloacal/oro-pharyngeal swab Blood	PCR	Isolation of the bird. After three negative tests within 3 months, birds can reincorporate to the breeding program.
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¹ If paramyxovirus is detected, it is important to identify its type (isolation and viral characterization), because not all of them are pathogenic to psittacines and only APMV-1 is notifiable. Considering Brazilian legislation, positive cases cannot be imported to Brazil.

² Birds tested positive for these viruses cannot be imported to Brazil.

Additional Observations:

- ❖ Feces should never be collected from the ground or from an unclean surface.
- ❖ Caution on interpretation of laboratory results. Positive results must be analyzed by veterinarians and associated with clinical signs and retests (counter-proof).
- ❖ Positive animals must be isolated from others during treatment, when necessary. The cage or aviary must be cleaned and disinfected properly, even with removal of plants (it may be necessary to use a blow torch on sand or cement floors).

Currently, the following lab tests are not available in Brazil: serology for Alpha-herpesvirus of Pacheco (PDV), Polyomavirus (APPV) and Avian Bornavirus. All other tests are available in Brazilian labs.

Additional assessment may include endoscopy, electrocardiogram, radiographs and semen sampling.

Electro-ejaculation should only be used when pairs are showing signs of breeding, as during this period the maximum amount of semen will be obtained. Outside of the breeding season males do not produce significant semen, including proven breeders. A single sample with low quality semen should not be used to determine whether or not a male is a bad semen donor, as it may have been caused by many reasons such as recent semen deposition into the female or asynchronization with female and thus not sexually active. It may be necessary to take several independent semen samples to permit a solid evaluation of the sperm from a given male. Especially if the semen quality is repetitively low, further veterinary examinations could help assess the male's fertility. In contrast, one or more samples with good quality could judge the male as good semen donor, at that current time.

Pairs that produced only infertile eggs in the past can be tested before and during oviposition. Males can be checked if they produce adequate sperm at the right time. If he produces semen, artificial insemination (AI) could be performed. Otherwise, a sample from another previously selected semen donor could be used.

AI can be performed with semen of the female's own partner or from another male. Semen collections should be primarily focused on the pairs with infertile clutches or bad fertilization rates in the past. If it is possible to collect adequate semen from problem pairs without disturbing the natural breeding pairs then those birds should be the pairs of primary focus, the proven pairs with breeding success should not to be used unless there is no alternative, as the continual catching could decrease breeding success in the proven pairs. If it is crucial to take semen of proven breeder males, it should only be performed after the clutch is complete and all eggs are laid. So there is no loss of sperm for the natural fertilization of the eggs.

To maximize success of AI, the maximum volume of the semen samples should be used for inseminations. The samples can be examined in capillaries to estimate the volume, assess motility and concentration without wasting too much sperm. If there are excellent samples, AI is not possible or semen collection is performed for investigation of the male's fertility, a detailed sperm examination can be performed.

It is also imperative to take blood or tissue samples for DNA analysis (send to Cristina's Miyaki lab) and for depositing at Genome Resource Banks/Frozen Zoos whenever indicated (not previously done). For DNA analysis 0.1 ml of blood can be stored in 0.5 ml of 100% ethanol without refrigeration.

4.7. Routine treatments

- ❖ **Parasite control:** parasite control should be part of an integral parasite program including regular parasitological analysis and appropriate deworming using the most indicated drugs and dosages, only when the birds are positive.
- ❖ **Aspergillosis prophylaxis:** few cases of aspergillosis have been reported in Spix's macaw but the risk depends mainly on environmental conditions at the holder. Seeds should be carefully inspected (see item 4.4), as they should be clean to minimize the risk of aspergillosis and disinfection when required.

4.8. Individual treatment

Except in emergency cases, treatment for sick or injured Spix's macaws can only be applied after a conclusive diagnosis is reached by the holding institution's veterinarian. If indicated, Spix's macaws must receive stationary treatment at the holding institution's clinic. In case of infectious diseases, the bird must be isolated.

4.9. Necropsies and storage of tissues and carcasses

A thorough necropsy shortly after death is extremely important to determine the cause of death. It may indicate health and/or management problems that may have gone unnoticed. Necropsies must be conducted on all birds that die.

Tissue sections must be carried out, and any pathological signals must be recorded. Samples should be taken for detection and identification of bacteria, fungi, parasites and viruses as well as for cytology and histopathology.

Tissue fragments for histopathological analyses should be preferably collected as cubes of 1 cm³, not exceeding 1 cm in any dimension, preserved in 10 % formaldehyde (proportion 1:9) and sent to the laboratory. Fragments collected for virus diagnosis and bird genetics should be frozen (-80°C is preferable).

Except for Cestoda, most endoparasites should be preserved in alcohol acetic acid solution (AFA) or Raylliet-Henry solution and ectoparasites should be preserved in 70° ethanol and both sent to the laboratory.

Material for microbiological analyses should be collected with sterile swabs (with or without culture medium) and sent to the laboratory. Feces samples should be preserved and sent to the laboratory. Body fluids (exudate or transudate) should be collected with sterile syringes and needles and sent to the laboratory.

Blood smears have to be prepared as soon as possible after blood collection and fixed in methanol.

All samples collected for examination (blood, feces, parasites, tissue, etc.) must be clearly labeled with the birds' identification and a sequential number. The accompanying record sheet must specify type and origin (organ) of each individual sample. Samples must be stored in adequate conditions.

After necropsy, the carcass should be kept in 70° ethanol or frozen. Both skin and carcass must be sent to an ornithological collection authorized by ICMBio.

If the necropsy results show evidence for any disease and are able to explain the cause of death, appropriate medical and management measures (prophylaxis and treatment) must be taken immediately (according to the type of disease and on the basis of the bird's transfer history and pairing/breeding history):

- ❖ for the current partner
- ❖ for other birds that may have been in contact with the dead bird including offspring and past partners, both on-site and off-site
- ❖ for the local macaw population
- ❖ for the population from former facilities where the bird was

❖ for the global population.

An immediate report must be always sent to the Coordinator and Management Consultant of the Captive Program in cases when a severe disease or pathogen is identified.

4.10. Control of neonates and the hand-rearing station

Young parrot chicks are susceptible to pathogens during their first days of life, because their immune system is not yet entirely developed. Currently, the main infection is *Candida* spp (Crosta et al., 2003). At the nursery module, both natural and incubator-hatched chicks must be raised in isolation from other birds to reduce the risk of infection with potential pathogens and concurrent disease spread.

Naturally-hatched chicks that enter the hand-rearing station should be placed in a separate *parents-hatched hand-rearing room* and should be immediately sampled for bacteriological and mycological analyses. The most common infections of hand-reared chicks are bacteriological (usually Enterobacteriaceae or other Gram negatives) and fungal (usually *Candida* sp).

Chicks must be constantly monitored for their health status, by assessing the following indicators:

- ❖ Normal development (weight, growth) as compared to standard growth curves (Appendix III)
- ❖ Normal posture
- ❖ Regular body proportions (not stunted)
- ❖ Other bone malformation evidences (including “constricted toe syndrome”)
- ❖ Hydration status
- ❖ Skin color, texture and fat deposits
- ❖ Normal feather development
- ❖ Normal shape of the beak (check for symmetry, prognathism or brachygnathism)
- ❖ Tone and thickness of the crop
- ❖ Presence of foreign bodies (*e.g.* sawdust) in the crop
- ❖ Feeding response
- ❖ Normal production and aspect of feces

Treatment or intervention must be applied and performed as indicated. An example of hand-rearing records is found in Appendix I.

4.11. Quarantine measures

Every bird that enters a given breeding facility must undergo a period of at least 30 days of strict isolated quarantine to preclude the spread of pathogens to the rest of the population/birds on site. If the bird becomes sick within these first 30 days, the quarantine period must start again when the bird is declared healthy again. Birds in quarantine have to be considered as Quarantine units, not individual birds. Treatment against intestinal parasites may be provided for birds undergoing quarantine if they are positive for parasite eggs or oocysts. All birds have to produce at least three negative parasites fecal samples within an interval of 7 to 14 days in the quarantine. Different tests should be performed according to the exporter and importer institution or country’s regulations. The procedures are detailed in the next sections.

Lab tests for the pathogens listed in item 4.6. must be performed on both holders (dispatcher and recipient) during the period of isolation. If the birds of one institution are considered free of diseases tested in item 4.6. it should only receive pathogen free birds or maintain positive birds separately (at least 10 m away or with a physical barrier, and equipment and handlers should be separated).

5. ENRICHMENT

Animals under human care should be: (1) free from thirst, hunger and malnutrition, (2) free from pain, injury and disease, (3) free of discomfort, (4) free to express natural behavior and (5) free of fear and distress (Animal Welfare Council). Failure to meet the needs of animals in captivity may result in compromised welfare, which may be presented as abnormal behaviors and/or diseases that could lead to

death. Some of the reasons that undesirable behavioral and physiological changes are triggered include boredom, fear, inappropriate social company, lack of shelter, among others.

Enrichment is a dynamic process for enhancing animal environments giving them the chance to choose or control their environment, taking into consideration their behavioral biology and natural history. At the individual level, the implementation of environmental enrichment programs can prevent and reverse welfare problems. At the population level, it may greatly improve conservation goals by allowing for an increase in reproductive rates and helping in the development of natural behavior patterns (especially important for reintroduction candidates).

Environmental enrichment programs should be goal oriented, tested and assessed frequently by a professional with expertise (just like veterinarians take care of birds health aspects). The Spix's macaws enrichment programs must aim to reach the needs of breeding animals, isolated, sick and/or retired birds, quarantine and release candidates (breeders may keep birds for other purposes that should also be considered). The use of planning tools or frameworks are highly recommended (e.g. http://www.enrichment.org/MiniWebs/About_EE/planning_chart.pdf)

In order to establish a program goals must be set by considering Spix's natural history and identifying desirable and undesirable behaviors that will be shaped through enrichment depending on the purpose of each individual in the program. Then, it is necessary to develop and implement the plan deciding which behaviors to encourage, whether or not the resources needed are available in your facility and how to substitute. There are 5 categories of environmental enrichment that should be contemplated in a monthly schedule: 1-food, 2-sensory, 3-social, 4-cognitive, 5-physical. It is very important to test for safety, document each phase and birds' responses so that the program can be evaluated and re-adjusted on a regular basis. The planning and results of each facility's program should be shared with other keepers and the specialist group so that an official Spix's macaw environmental enrichment program can be established.

Below are a few examples for each category:

1-Food:

- ❖ Variation of food items, including novel items that are part of their natural diet
- ❖ Food Presentation: puzzle feeders, food offered in different locations of the enclosure

2-Sensory

- ❖ Stimulation of the macaws' senses-touch (nest boxes, leaves, different substrates), olfactory and taste (novel food item, water presented in plants or as rain), hearing (vocalization of other species present in the natural habitat, including predators if training for reintroduction) and vision (moving objects, other individuals)

3-Social

- ❖ Promotion of interaction between conspecifics or different species found in their natural habitat
- ❖ People, keeper and visitors (for retired birds)
- ❖ Inanimate objects (stuffed toys, mirrors)

4-Cognitive

- ❖ Stimulation of cognitive abilities to explore and resolve situations such as puzzle feeder, predator or other species recognition
- ❖ Conditioning sessions
- ❖ Novel experiences

5-Physical

- ❖ Addition or modification of captive environment complexity, including of perches, plants, hiding areas, nests and nesting material, climate gradients such as light, rain, temperature
- ❖

6. PAIRING AND INSTITUTIONAL TRANSFER

The following criteria are set forth to determine which Spix's macaw holder will send and which one will receive a bird when a pairing recommendation is made involving birds from different institutions. Pairing recommendations are determined through an assessment by the studbook keeper and Management Consultant based on genetic importance and compatibility, reproductive potential, health status and demography. The final decision on whether a recommendation will be followed or not depends on the Brazilian Government and the negotiation between the owners of the birds.

- ❖ All pairing recommendations involving a proven breeding male: the female will be transferred to the holder institution of the male. As there are fewer proven breeding males in the studbook managed program, they should be safe-guarded as best as possible, which includes mitigating the risk of capture, handling and transportation injuries. An exception to this rule can be made when capture, handling and transportation risk is deemed greater for the female (e.g. a female that seizures during handling).
- ❖ All pairing recommendations that do not involve a proven breeding male: the studbook keeper will inform which of the two birds is reproductively more important to the population. This decision should be taken based on reproductive history, endoscopic analysis of gonads, spermatozoa analysis, genetic similarity value, mean kinship value and known pedigree. The bird assessed as being of lesser importance to the population should be the one transferred.

7. TRANSPORTATION

For the transit of wild live birds in Brazil a Transport Authorization (AT) from IBAMA or other state agencies is required. Additionally, for transit between states in Brazil the Animal Transit Guide (GTA) issued by the Brazilian Ministry of Agriculture, Livestock and Supply (through Decree No. 5741/2006). This official document contains information about destination, health conditions, and the purpose of animal transportation. For GTA, a health certificate issued by a certified veterinarian with CRMV from the country of origin is required. Information on the health certificate is found at:

http://www.agricultura.gov.br/arq_editor/file/Aniamal/MercadoInterno/transito/manual%20gta%20animais%20silvestres.pdf.

For international transit, CITES permits from the import and export countries are required. The authorization for the importation of live animals follows the 'Normative Instruction' MAPA n°. 17 from August 3rd 2010 (in review). The authorization has to be requested to the Federal Superintendent of MAPA in São Paulo (SFA-SP) as well as the schedule of quarantine. The birds arrive in Guarulhos or Campinas airport accompanied by the International Zoosanitary Certification, issued or endorsed by the competent health authority of the origin country, in which one language is Portuguese. Birds are intended for Quarantine Station of Cananéia (EQC) by the MAPA in acrylic bio boxes.

The country, zone or compartment of the birds' origin must comply with the Sanitary Code for Terrestrial Animals of the World Organization for Animal Health (OIE) to be considered officially free from notifiable Avian Influenza and Newcastle disease. The country of origin of birds should not have officially reported cases of Crimean Congo hemorrhagic fever and imported birds should not have been in an area within a radius of 10 km with officially reported cases of West Nile Fever were reported in the 90 days prior to boarding.

The institution or place of origin of birds should not have registered any clinical case of avian infectious laryngotracheitis, infectious bursal disease, avian infectious bronchitis, avian cholera, avian chlamydiosis, fowl pox, avian encephalomyelitis, equine infectious anemia and eastern equine encephalitis, and West Nile Virus over the past 90 days prior to importation into Brazil.

The birds must remain isolated for at least 30 days prior to shipment, under the supervision of an official veterinarian or authorized by the Official Veterinary Service of the country of origin. The birds should be subjected to diagnostic tests with negative results for Newcastle disease and avian influenza

(hemagglutination inhibition test - HI test or isolation and identification of the agent or other test recommended by the OIE and approved by the DAS) prior to transportation to Brazil.

The birds must undergo treatments against internal and external parasites during the quarantine period with products approved or recognized by the Official Veterinary Service of the exporting country.

The macaws should undergo diagnostic tests for chlamydiosis during the quarantine period, by testing for the detection of antigens of *Chlamydia psittaci* (ELISA or PCR) from conjunctival swabs, choanal, cloacal swabs or fresh feces, or other test recommended by the OIE and approved by DSA. In case of positive results, parrots should be treated for chlamydiosis with effective antimicrobial approved or recognized by Official Veterinary Service of the exporting country. Birds must be retested after treatment to certify its efficacy. The period between the end of isolation and shipment of birds cannot be more than five days.

During quarantine in Brazil, the imported birds should also be subjected to diagnostic tests for Newcastle disease and avian influenza (Quarantine Station of Cananéia). The exams have to be made in laboratories belonging to 'Rede Nacional de Laboratórios Agropecuários do Sistema Unificado de Atenção à Sanidade Agropecuária'. The birds are accompanied by a technical officer appointed by the importer, and are released from quarantine after fulfillment of the isolation period, confirming the negative diagnosis for pathogens monitored by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) and after its official authorization.

The notifiable diseases for parrots in Brazil are: Influenza and Newcastle. Salmonellosis (*S. enterica* Pullorum, Gallinarum, Typhimurium and Enteritidis) and mycoplasmosis (*M. gallisepticum*, *M. sinovae* and *M. melleagridis*) and are also notifiable for production and marketing animals.

In Germany laws are: Animal Welfare Act: Federal Law Gazette I, p. 1094 of May 25, 1998 and Regulation EC 1/2005 - protection of animals during transport. In Qatar IATA laws are valid.

Both holders (dispatcher and recipient) will designate a technician(s) responsible to monitor the transportation, with the supervision of a CEMAVE's technician in Brazil, from the place of origin to the final destination, according to the following:

- ❖ Birds should not be transferred during months of extreme weather conditions.
- ❖ All birds must be transported from the place of origin to the destination as directly and quickly as possible, with no contact to other animals.
- ❖ Birds should be transported with adequate ventilation and low noise level.
- ❖ Preferably, the airline chosen for transportation shall not carry other animals on the same flight as the one carrying Spix's macaws (also valid for other vehicles).
- ❖ Persons responsible for sending and receiving birds are obliged to keep the Spix's macaw Management Consultant and/or Coordinator informed of all developments concerning the transfer.
- ❖ The birds should always be carried in separate boxes constructed according to IATA standards. The crate can be made of plastic and must offer sufficient space and perches as well as bowls for food and water, and be furnished with a substrate (e.g. non-toxic digestible cat litter, sterile wood-shavings) on the floor of the carrier suitable for absorbing excrements and any water spillage. The containers must be disinfected before utilization and incinerated after use.
- ❖ The bird must be provided with sufficient food (pellets of a reliable quality, calculate 60-100 g per day plus spillage) and clean water provided prior the period of travel.
- ❖ The recipient institution must receive detailed information on the bird's medical, behavioral, reproductive and husbandry history from the dispatcher institution to facilitate a smooth adaptation at the new facility. The exporting institution has to provide copies of all the medical files (and any other important information available) to the importing institution.
- ❖ The dispatcher institution has to do pre-export testing for diseases as requested by the importing institution.
- ❖ After arrival at the destination, the bird will be isolated and quarantined according to the previously described protocol. After completing quarantine, the newly arrived bird must be introduced to his/her new partner or group in accordance to the intra-specific introduction protocol (item 7.2) to minimize the risk of rejection, aggression, injury and even death.

7.1 Brazilian Animal Transport Legislation

Additional laws for animal transportation by the Brazilian Ministry of Agriculture are:

- ❖ Portaria 193/1994 that institutes The National Health Poultry Program;
- ❖ Portaria 70/1994 that regulates mandatory communication of Newcastle disease suspicion;
- ❖ Instrução Normativa 17/2006 that approves, under The National Poultry Health Program, the National Avian Influenza Prevention and Control and Prevention of Newcastle Disease;
- ❖ Instrução Normativa 44/2001 that approves the Technical Standards for the Control and Certification for Avian Mycoplasmosis (*Mycoplasma gallisepticum*, *M. melleagridis* and *M. synoviae*); and

7.2 Protocol for intra-specific introductions

Appropriate care must be taken whenever birds are introduced to one another, to minimize the risk of aggressive attacks that can result in injuries or fatalities. This applies to introductions of:

- ❖ “Type 1”: the introduction of formerly unpaired or ungrouped individuals
- ❖ “Type 2”: the repairing or regrouping of birds previously together but temporarily separated (*e.g.* because of extended medical treatment).

For all such introductions, the steps bellow can be used as a guide:

- ❖ Place birds in adjoining aviaries with full visual contact and the possibility to establish close contact through the double wire mesh. As an alternative, put the newly arrived or returned bird(s) in a small (*e.g.* 1 x 1 m) wire cage and hang or place this cage at the outside or inside of the aviary of the residential birds(s). In the case of a new pairing, it is preferable (but not essential) that the new pair be formed in the aviary of the female.
- ❖ Closely observe the tolerance and compatibility of the birds to be introduced to one another and evaluate the level of (potential) aggression. If birds appear compatible (*i.e.* no indication of aggression) proceed with the introduction. Should aggressive behavior be observed during this adaptation period, the birds should not be paired or flocked.
- ❖ To complete the introduction, either of the following applies:
 - release the birds together into their aviary at the same time and during the morning hours; in the case of a newly determined pair, transfer to a new breeding aviary; this technique is preferable for “Type 1” introductions, to eliminate any previous territorial occupation.
 - introduce the newly arrived or returned bird(s) to the aviary of the bird(s) already previously on site (resident), during the morning hours; this technique is preferable for “Type 2” introductions, but can also be applied for “Type 1” introductions.

From the moment of physical introduction, keepers and/or curator staff must closely monitor the birds throughout the day and always be ready and equipped to immediately separate the birds should severe aggression occur. If there is no severe aggression observed on the first day, it is highly unlikely that incompatibility is going to be a problem and therefore future monitoring can follow those outlined in the husbandry protocol.

8. REINTRODUCTION

This protocol will be prepared in the next years, when more information is available, after the building of the Breeding and Reintroduction Center in the Caatinga. First of all we established the categorical ages of Spix’s macaw as:

- ❖ Chick: from pip in the egg till weaned (age of weaning ~ 70 - 90 days old)
- ❖ Juvenile: from weaning till sexually mature ~ 3 years old
- ❖ Adult: once sexually mature (~ 3 years old)

Reintroduction age of the Spix’s macaw, after weaning, the best period would be within the first year, preferably 4 - 9 months old. Timing is crucial, and the fledgling of wild Blue-winged macaws should mark the best time to release into the wild. This will affect the release age more than the age of the birds for reintroduction (as long as the release birds are weaned and had the necessary training).

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Appendix II - Formula chart with an indication of the feeding percentages followed by AWWP



AL WABRA WILDLIFE PRESERVATION

Sheikh Saoud Bin Mohd. Bin Ali Al-Thani

Fluids	10 gr total	20 gr total	30 gr total	40 gr total	50 gr total	60 gr total	70 gr total	80 gr total	90 gr total	100 gr total	130 gr total	160 gr total
Solids												
10 %	9 1	18 2	27 3	36 4	45 5	54 6	63 7	72 8	81 9	90 10	117 13	144 16
12 %	8.8 1.2	17.6 2.4	26.4 3.6	35.2 4.8	44 6	52.8 7.2	61.6 8.4	70.4 9.6	79.2 10.8	88 12	114.4 15.6	140.8 19.2
14 %	8.6 1.4	17.2 2.8	25.8 4.2	34.4 5.6	43 7	51.6 8.4	60.2 9.8	68.8 11.2	77.4 12.6	86 14	111.8 18.2	137.6 22.4
15 %	8.5 1.5	17 3	25.5 4.5	34 6	42.5 7.5	51 9	59.5 10.5	68 12	76.5 13.5	85 15	110.5 19.5	136 24
16 %	8.4 1.6	16.8 3.2	25.2 4.8	33.6 6.4	42 8	50.4 9.6	58.8 11.2	67.2 12.8	75.6 14.4	84 16	109.2 20.8	134.4 25.6
18 %	8.2 1.8	16.4 3.6	24.6 5.4	32.8 7.2	41 9	49.2 10.8	57.4 12.6	65.6 14.4	73.8 16.2	82 18	106.6 23.4	131.2 28.8
20 %	8 2	16 4	24 6	32 8	40 10	48 12	56 14	64 16	72 18	80 20	104 26	128 32
22 %	7.8 2.2	15.6 4.4	23.4 6.6	31.2 8.8	39 11	46.8 13.2	54.6 15.4	62.4 17.6	70.2 19.8	78 22	101.4 28.6	124.8 35.2
25 %	7.5 2.5	15 5	22.5 7.5	30 10	37.5 12.5	45 15	52.5 17.5	60 20	67.5 22.5	75 25	97.5 32.5	120 40
27 %	7.3 2.7	14.6 5.4	21.9 8.1	29.2 10.8	36.5 13.5	43.8 16.2	51.1 18.9	58.4 21.6	65.7 24.3	73 27	94.9 35.1	116.8 43.2
28 %	7.2 2.8	14.4 5.6	21.6 8.4	28.8 11.2	36 14	43.2 16.8	49.4 20.6	57.6 22.4	64.8 25.2	72 28	93.6 36.4	115.2 44.8
30 %	7 3	14 6	21 9	28 12	35 15	42 18	49 21	56 24	63 27	70 30	91 39	112 48

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APPENDIX III – Normal development (weight mean and standard deviation) of young Spix's Macaws between 1 and 150 days old based in standard growth curves of Al Wabra Wildlife Preservation chicks born in the period between 2006 and 2011 (Groffen *et al.* 2008 and Tschudin *et al.* 2010)

Days old	Mean	SD	Days old	Mean	SD	Days Old	Mean	SD
1	13,1174	1,366283	51	342,652	18,02425	101	288,087	14,78302
2	14,2343	1,845025	52	343,043	17,96835	102	288,174	14,26264
3	16,493	1,954069	53	342,522	18,80732	103	288,739	14,88507
4	19,6557	2,631074	54	342,043	18,55331	104	287,391	15,16497
5	24,0122	3,412052	55	340,696	17,37405	105	286,391	14,87139
6	28,9335	3,764729	56	339,435	17,2174	106	287,348	16,54912
7	35,0087	4,608482	57	337,565	16,9701	107	285,13	15,97812
8	41,6348	5,457239	58	336,348	17,21154	108	285,826	16,64237
9	49,2522	6,276335	59	334,913	15,90573	109	286,087	15,91144
10	57,3609	7,547049	60	332,739	16,04317	110	286,591	15,59505
11	65,5609	7,675447	61	330,957	15,91305	111	286,682	15,25462
12	73,9783	8,191956	62	327,391	15,64591	112	285,727	14,54118
13	84,087	8,732505	63	325,174	16,83517	113	287,227	14,46155
14	93,2826	8,926653	64	322,478	17,63743	114	286,909	13,8767
15	103,946	9,771956	65	319,957	18,08434	115	288,227	13,54686
16	114,865	9,17564	66	318,087	17,60659	116	288,19	14,56921
17	125,313	9,12396	67	315,826	17,78317	117	287,762	14,20178
18	135,963	9,870597	68	312,348	16,99628	118	287,895	15,94398
19	147,174	11,47405	69	310,696	17,66979	119	288,895	16,18262
20	158,374	11,59499	70	306,913	16,84397	120	286,947	17,44666
21	169,826	10,52984	71	304,522	17,75815	121	287,421	17,73357
22	181,261	12,5235	72	302,087	17,43537	122	287,947	17,84026
23	192,348	12,179	73	300,609	16,01346	123	289,474	18,70344
24	203,304	13,48209	74	298,478	16,44238	124	289,211	17,75944
25	214,957	13,66641	75	296	16,92497	125	287,684	18,99431
26	228,217	13,14242	76	294,304	15,83849	126	287,316	17,57856
27	237,913	13,372	77	293,913	16,80885	127	287,579	17,86777
28	248,174	13,79279	78	292,391	16,92964	128	287,526	18,27983
29	260,478	15,64629	79	291,696	16,99849	129	287,316	17,05889
30	271,043	14,67985	80	290,087	15,77948	130	288,5	17,40943
31	279,304	14,81226	81	290,826	16,5135	131	287,833	17,17471
32	288,696	15,69723	82	289,13	16,26291	132	288,333	17,41872
33	297,174	16,63417	83	289,913	16,81156	133	289,278	16,5238
34	305,435	17,03426	84	288,348	16,2861	134	291,722	17,50901
35	311,826	17,47635	85	288,174	17,35368	135	292	16,99827
36	317,087	16,88171	86	288,13	17,30293	136	294,056	16,44768
37	323,826	16,46388	87	289,522	19,25633	137	290,167	18,76558
38	327,913	16,18055	88	288,522	18,24049	138	290,5	17,0337
39	330,087	16,42867	89	289,696	18,98189	139	289,389	16,26667
40	333,522	19,39744	90	289,348	17,48404	140	289,556	17,7241
41	336,174	19,90581	91	286,304	18,27426	141	288,778	16,92042
42	336,391	18,43105	92	286,739	17,21579	142	289,5	16,35003
43	339,739	19,67975	93	287,522	15,75053	143	288,833	15,48909
44	340,87	19,16508	94	286,217	15,61746	144	287,389	15,09242
45	342,478	20,21446	95	286,348	14,47446	145	286,889	15,49151
46	343,087	19,62585	96	287,522	15,25501	146	285,833	14,18056
47	343,696	18,61191	97	288,478	14,30152	147	287,056	16,81901
48	344,043	18,47967	98	288,522	14,28562	148	286,278	14,63198
49	344,391	16,74607	99	287,87	14,86088	149	287,667	15,3508
50	343,87	17,78739	100	288,478	14,38708	150	286,889	16,06929