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SAFE FIELD TECHNIQUES FOR NEST INSPECTIONS AND NESTLING CROP SAMPLING OF PARROTS

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Abstract.—During 1991–1997, we developed, adapted, and tested a variety of field techniques for the study of *Amazona* parrots in northeastern Mexico. We describe two of these techniques considered novel and employed for nest inspections and sampling crop contents of nestlings. Inspections included modification of nests and use of a video probe. Crop sampling of chicks was done using a plastic cylinder from 20 days of age until fledging. The techniques did not cause nest abandonment or increase in chick mortality. We obtained information that has substantially advanced understanding of the biology and conservation needs of these species. We hope these techniques will aid future studies and facilitate comparisons among diverse species.

TÉCNICAS DE CAMPO SEGURAS PARA LA INSPECCIÓN DE NIDOS Y TOMA DE MUESTRAS DEL BUCHE DE LOROS

Sinopsis.—Durante 1991–1997, desarrollamos, adaptamos, y probamos una variedad de técnicas de campo para el estudio de loros *Amazona* en el Noreste de México. Describimos dos de estas técnicas de investigación: (1) Inspección de nidos, y (2) Muestreo del buche de los pichones. La inspección incluyó acondicionamiento de nidos y uso de una sonda de video. Las muestras de buche fueron en pichones a partir de 20 días de edad y hasta el vuelo utilizando un cilindro de plástico. No se causó abandono de nido o mortalidad adicional con estas técnicas. Obtuvimos información que ha avanzado sustancialmente nuestro entendimiento de la biología y necesidades de conservación de estas especies. Esperamos que estas técnicas sean de ayuda en estudios futuros y faciliten la comparación entre distintas especies y estudios.

More than one third of New World parrot species are threatened with extinction by a combination of natural and anthropogenic processes (Collar and Juniper 1992). Deforestation and capture for the pet trade are the two most important threats (Nilsson 1989, Beissinger and Snyder 1992, Collar and Juniper 1992), but a variety of other forces threaten some species. Because basic data on parrot biology are needed to design effective conservation and management actions for particular species and regions, many studies on psittacines have been initiated, mostly in Australia (e.g., Saunders 1982, Saunders et al. 1982, Rowley 1983, Smith and Saunders 1986).

Until 1990, most of the in-depth research on Neotropical parrots re-

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sulted from an intensive effort to save the Puerto Rican Parrot (*Amazona vittata*) from extinction (Snyder et al. 1987). In more recent years a number of other projects have been started in the Neotropics. As a result of this increased interest in psittacines a number of field techniques have been published recently, still largely from work in Puerto Rico, concerning field methods such as radio tracking (Lindsey and Arendt 1991), tree-climbing (Munn 1991), nest guarding (Lindsey 1992), capture using nets (Meyers 1994a), PVC color bands (Meyers 1994b), radio-collars (Meyers 1994c, 1995), and nest observations (Wilson et al. 1995).

In 1990, we started a comparative study of three sympatric *Amazona* parrots in southern Tamaulipas, Mexico (Red-lored Parrot, *A. autumnalis*; Yellow-headed Parrot, *A. oratrix*;, and Red-crowned Parrot *A. viridigenalis*, $n = 46$ nests). In the course of our study we developed, tested, adapted and, in some cases, discarded a variety of techniques. Among the field techniques tested were procedures for (1) measurements of cavity suitability and availability, (2) nest searches, (3) intensive nest observations, (4) nest modifications for sampling, (5) intensive manipulations for nestling mass and measurements, (6) individual identification through high-magnification video, (7) behavioral observation through high-magnification video, (8) attachment of radio collars, and (9) roost counts and population indices to be presented elsewhere (Enkerlin-Hoeflich 1995, unpubl. data). Two procedures represent largely new approaches that have yielded especially valuable information when applied to the study of *Amazona* parrots: (1) nest inspections using a burrow probe, and (2) sampling of nestling crops. These procedures may prove applicable to a variety parrots as well as other species and deserve some detailed discussion. We hope this paper will aid researchers in obtaining information that may be comparable across studies and taxa, and may provide a sound basis for effective conservation and management efforts.

DESCRIPTION AND DISCUSSION OF TECHNIQUES

The study for which most of these techniques were used was conducted on a working cattle ranch in southeastern Tamaulipas, Mexico (22°55′N, 97°49′W; Pérez and Eguiarte 1989) from 1991–1997. Disturbed vegetation types (83% pastures with remnant trees, Enkerlin-Hoeflich et al. 1992) dominated the 526-ha ranch. The ranch was reasonably typical (though slightly more wooded) of habitats available to parrots in the region (Enkerlin-Hoeflich and Packard, unpubl. data). The principal rainy season occurs in late summer or early fall; a secondary rainy season sometimes occurs in spring. A more complete description of the study area is presented elsewhere (Enkerlin-Hoeflich 1995).

Intensive nest inspections and use of video probe.—Sample size is often the most important limiting factor in analyses of mortality factors and productivity in wild parrots because the presence and number of eggs and chicks in nests can only be confirmed with difficulty via visual inspection. For cavity-nesting species, nests are frequently too deep and dark or have curves in the cavity, precluding reliable observation of the

full contents from cavity entrances. Such was the case for over 60% of *Amazona* parrot nests in Tamaulipas. To overcome this problem, we experimented with several known methodologies such as flashlights, flashlights with mirrors, periscopes, and endoscopes inserted through a small hole in the sides of the nest trees. Success was limited until we began using a "burrow probe" (Burrow Probe 3, Fuhrman Diversified Inc., LaPorte, Texas) consisting of a video imager with infrared illuminator on a long flexible cord. The burrow probe allowed routine inspections of nests as deep as 8 m and allowed us to achieve significant improvements in the quantity and quality of data obtained. The burrow probe, by providing us with unequivocal information on nest contents, also allowed us to plan allocation of our sampling efforts and to decide which nests to safely modify for some of our more intensive sampling techniques. Using the probe in association with a video recording device for behavioral studies allowed study of some of the ultimate causes regulating productivity (Schindlinger and Enkerlin, unpubl. data).

Nest inspections with or without the probe were normally conducted twice a week, except during egg-laying and hatching, when they were conducted every 2 d to determine accurately incubation period and timing of hatching. During the incubation and early nestling phases, logistical considerations did not allow us to restrict nest inspection to the times when the females were outside of their nests, and inspections were conducted during the activity lull in the middle of the day. Females usually left the nest upon our arrival or were coaxed out by gentle tapping on the nest tree trunk (Enkerlin-Hoeflich 1995). However, some females could not be coaxed out of their nest cavities and special inspections had to be made when they were away naturally as part of their daily routine. Despite our initial fears that nest inspections might cause some birds to abandon nests, no case of nest desertion (out of 123 nests examined) could reasonably be attributed to the inspections.

To access nest entrances we usually used aluminum ladders. This was possible because of the open habitat and short height of trees in the areas in which we conducted our studies. For certain nests, far away from roads or not accessible with a ladder, we expect in the future to use "pole-steps" (L-shaped metal screw-in pieces such as used at times in electric and telephone poles). For high nests (15 m or more) tree-climbing equipment is often advisable (Munn 1991; N.F.R. Snyder, pers. comm.).

To allow convenient handling of eggs and chicks in natural nest cavities, some nests were fitted with "portholes" by cutting a 12 × 12-cm square opening through the sides of the trees close to the bottoms of the cavities. The opening was normally cut using a chain saw, which was easiest and faster than other methods, usually before the start of the breeding season. We found that it was best to make the cuts slightly converging such that the piece of trunk removed could be used as a tight porthole cover to maintain crypticity and prevent entry of light. It is important to make sure that the appropriate location for the cut is measured to permit access to the nest contents without being too close or too far from the cavity

bottom. The burrow probe is useful in making this decision and also making sure that an incubating birds is not present in the cavity. Selection of the presumed thinnest wall is also recommended. The best time to cut the porthole is before eggs are laid but when there are behavioral cues to suggest that the cavity will be used. If this is not possible it should be done as early as possible during egg laying.

At the onset of the study, we were cautious in continuing observations and measurements after six weeks of age, as certain *Amazona* parrots, in particular *A. vittata,* have been reported to become sensitive to handling at this stage (Snyder et al. 1987). Fortunately no indications of stress became apparent at any time during our studies, and chick handling continued uninterrupted until fledging. This finding revealed important interspecific differences in sensitivity even within the genus *Amazona.* We emphasize the need to always proceed slowly and incrementally in developing any handling program for birds, with careful attention to tolerance levels at all times.

Extracting crop contents from Amazona parrot nestlings.—As has been traditional in many parrot field studies (Desenne 1994, Enkerlin-Hoeflich 1995, Martuscelli 1995), we also made opportunistic observations of feeding parrots during field operations in 1992–1997. Nevertheless, daily palpation of food in chick crops was part of the intensive chick monitoring described above, and limited sampling of chick crop contents by regurgitation indicated that food consumed by chicks differed from that observed in foraging adults. It was clear that to understand what parrots were feeding their chicks, we would have to sample chick crops directly.

Based on behavioral information acquired during the nesting period of 1992, we had decided to closely monitor chick growth and food provisioning at two nests of *A. viridigenalis,* in 1993 and 1994. We gained enough confidence to initiate crop sampling and intensive weighing of the three *Amazona* species in a regular fashion in 1995, 1996, and 1997 (Enkerlin-Hoeflich, unpubl. data).

Despite some success in 1993 in extracting crop samples, we still lacked a consistent and safe methodology to extract routinely and quickly adequate crop samples for analysis. The main difficulty in extracting crop contents from wild chicks was that most of the contents were large chunks of food including whole seeds (Enkerlin-Hoeflich and Hogan 1997, Enkerlin-Hoeflich, unpubl. data). Collection of whole seeds from the crop is difficult and can potentially injure the bird.

To overcome these problems, we developed a sampling tube made from the cylinder of a standard plastic syringe from which we had cut off all the front part, leaving an opening as wide as the full cylinder diameter at the end. The front is cut off and then the sharp end of the cylinder was flamed over a gas burner (or cigarette lighter) for approximately 2 s to round the edges. The sampling tube can then be inserted into the chick's mouth on the left side of the bill and oriented towards the right side of the back of the mouth and lowered through the esophagus into the crop. We initially used a drop of mineral oil to lubricate passage of

the sampling cylinder but now use only water. The chick must be held gently but firmly, as it will react to the invasive process. This is best done with the left-hand (for right-handed persons) using the palm and little and ring fingers. The head is held with the neck slightly relaxed using the thumb, index, and middle fingers. A single person with experience can perform the full process but we recommend two people: One person can control the body of the chick and the other manipulate the head, syringe and massage the crop. We have found that if the chick can grasp a small stick, or even the loose clothes of the researcher with its feet, it will tolerate handling better.

Once the syringe has been inserted into the crop, we massaged the crop, working the contents towards the entrance of the cylinder. We first used the plunger from the original syringe to exert a negative pressure and extract the contents by suction. This worked well but we encountered problems in getting biased samples because the fluid part of the crop contents tended to come out easier than the solid part. We eventually learned that is actually easier, faster, less stressful to the birds, and less biased if we only insert the cylinder, without the plunger, and gently massage the crop to get the contents into the cylinder. Sometimes one can actually feel the seeds and the sides of the cylinder and can direct them into the cylinder. We do this until the contents reach the top of the cylinder or when judgment is made that the bird is uncomfortable (i.e., tries to move away or jerks its neck or pushes away).

It is usually possible to extract a sample in less than one minute (10–15 s is most usual) without apparent stress to the chick. We do not recommend handling for more than two minutes.

We started sampling crops as soon as we are able to introduce a 3-cc cylinder (outside diameter 11.5 mm) into a chick's crop at about 21 days of age. At this age, and occasionally earlier, we have been able to safely sample *Amazona viridigenalis,* for which adults weigh about 300 g, *A. autumnalis,* for which adults weigh about 360 g and *A. oratrix* for which adults weigh about 450–500 g. For the latter two species, we have been able to shift to sampling with a 5-cc cylinder (14.0 mm outside diameter) after about 28 days of age. We do not recommend using a 5-cc cylinder for *A. viridigenalis* at any age because it is too large to introduce safely into the oral cavity. Thus, we do not recommend using the 5-cc cylinder size, or equivalent, for *Amazona* parrots of about 300 g adult mass or less such as Hispaniolan (*A. ventralis*), Puerto Rican, White-fronted (*A. albifrons*) or Yellow-lored (*A. xantholora*) Parrots. If width of lower mandible is at least 13 mm, a 3-cc cylinder can be considered safe. Five *A. agilis,* the smallest *Amazona* parrot, were successfully sampled by EEH in 1996 at about 25 days of age using a 3-cc cylinder.

Some of the advantages of this method are that it allows relatively unbiased sampling and quantitatively adequate sample volumes, even when crops contain diverse food items. Each sample from a chick may represent a multitude of feeding activities by the adult birds. Seeds are generally delivered whole to the chicks and can be readily identified. Prior to our

crop sampling efforts, we had assumed that parrots might in general macerate their food as they ingest it to help their young in the digestion process, but this has not proved to be the case.

Sampling should be made as soon as possible after food delivery by adults for several reasons including the fact that (1) the food items are closest in humidity and integrity to the state in which they were delivered, (2) this maximizes the volume available for sampling, and (3) the chicks seem calmest at this time. We are not currently replacing the amount of food extracted with an equivalent amount of artificial or natural diet. The small amounts taken, even under repeated sampling, have represented less than 5% and usually less than 2% of the total food received during the nestling cycle (Enkerlin-Hoeflich, unpubl. data). Addition of replacement food could introduce additional sources of variability and possibly alter parent and or chick behavior and preferences.

As a general rule we do not sample chicks unless they have a crop content judged to be more than "25% full" and in this case take only a single cylinders worth (i.e., 3 or 5 cc). For chicks with more than "50% full" crops we take two cylinders worth of sample (i.e., 6 or 10 cc). Samples are currently being put in plastic bags and frozen for later analysis but we would recommend, if logistics permit, that food items be separated and weighed immediately, using a scale accurate to 0.01 g.

We have attempted sampling chicks less than 21 d old with a sampling tube made from a 1-cc syringe, but even at an early age the chicks seem to be receiving large chunks of food that are hard to withdraw with a small cylinder without bias. We presently do not recommend sampling of chicks younger than 2 wk, because of such biases and inadequate sample sizes, as well as possible risks to the birds. We are currently testing a tube larger in diameter than a 1-cc syringe but potentially safe for sampling chicks 14–21 days of age.

While the whole process of sampling seems rough, we have not observed that it has caused any harm to the birds. We have taken samples from over 150 chicks, several of which have been sampled more than 25 times, yet survival probabilities using Mayfield method (Johnson 1979) and growth rates have not differed statistically for sampled versus non-sampled chicks (Table 1). To date there have been no mortalities during handling and we have not noted malformations or bruises to the bill that might cause the birds future problems. In only three deaths, of the more than 150 birds sampled, we could not fully eliminate crop sampling as the potential cause of death, but neither could we conclude that it was the cause. Yet when seen in context of general mortality the technique emerges as extremely safe, when properly conducted. In 1995, we collected 234 samples. We considered chicks to be under the effect of sampling during the 48 hours immediately after sampling. Six chicks died during this period, three clearly due to other causes, and the three mentioned to unidentified causes. In 1996, when we took 364 samples, the causes of mortality were determined for all the birds dying during the period of crop sampling. In general mortality was considerably lower than

in 1995 and none was related to handling or crop sampling. Considering all cases of mortality in 1995 and 1996, the Mayfield estimate of survival for the "crop-sampled population" (Johnson 1979) was actually smaller although statistically the same as the controls. During 1997, 352 crop samples were collected, mortality per chick/day in the crop-sampled population was again lower than in the non-sampled population; Mayfield estimates were not calculated in 1997.

To have an alternative measure of potential effects of our crop sampling and intensive monitoring on chicks, other than handling-induced mortality; we compared four development parameters among intensively monitored (daily checks) and non-intensively monitored nests (about twice weekly checks) in a pooled sample of 1995, 1996 and 1997 breeding seasons (Table 1). Although there were differences in (1) maximum mass attained, (2) age at which maximum mass attained, and (3) last mass before fledging; none were significant. In the case of age at fledging, the two species with reasonably large sample size yielded highly significant differences in fledging age for the two treatments. We presume that our handling might have elicited an earlier fledging due to a combination of several factors. Secondly, we may have induced earlier fledging by reducing amounts of food delivered in the 2 wk before fledging. Even though no increased mortality or developmental problems seemed associated with our techniques, it is possible that the "quality" of chicks could be reduced as a result of handling and earlier fledging. We have no way of documenting how important this could be in overall chick survivability post-fledging but we suspect such an effect, if present, would be minor.

The techniques described have been slowly developed as we gained confidence in method safety. The population we worked with has been under study for six years and may be habituated to some extent to our presence and handling. Some individuals or species may be more sensitive than others. Thus, while we encourage parrot biologists to try to get the most information during field studies, we also caution that the techniques that worked for us may not be applicable to other parrots.

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TABLE 1. Comparison of four development parameters between intensively manipulated and not-intensively manipulated *Amazona* parrot chicks of three species in Tamaulipas, México during 1995–1997.

Species	*Amazona autumnalis*			*Amazona oratrix*			*Amazona viridigenalis*		
	Intensive, $n = 10$	Non-intensive, $n = 13$	P[a]	Intensive, $n = 4$	Non-intensive, $n = 3$	P[a]	Intensive, $n = 25$	Non-intensive, $n = 24$	P[a]
Mean maximum mass attained	386.76	404.81	0.40	486.15	464.30	0.32	335.30	325.55	0.28
Mean age at which maximum mass attained	41.30	39.80	0.51	38.25	34.33	0.19	37.00	36.67	0.79
Mean last mass before fledging	333.85	359.35	0.26	405.95	390.53	0.42	288.90	291.02	0.45
Mean age at fledging	53.11	56.86	<0.001	56.25	55.33	0.64	52.05	54.68	0.01

[a] *t*-test.

guay, ECEH had the opportunity to share and receive feedback on some of this information, thanks to all parrot biologists attending and especially to Phillipe Desenne. We are sincerely grateful to the Clynes family owners of the site and facilities (Rancho Los Colorados) where most of the information was collected and to all ranch personnel. Primary funding was provided in 1992–94 by the Mexico Program at World Wildlife Fund-US and TRAFFIC-USA. ECEH is also thankful to the Consejo Nacional de Ciencia y Tecnologia (CONACYT) of Mexico for a two-year fellowship to complete his Ph.D. Funding for diet studies in 1994–97 was kindly provided by Kaytee Products, Inc. and CSTB. Additional funding in 1995 was provided by CONABIO (Comision Nacional para el Estudio y Uso de la Biodiversidad de México). To Mexican wildlife authorities at Direccion General de Aprovechamiento Ecologico de los Recursos Naturales of SEDUE (1992 and 1993), SEDESOL (1994 and 1995) and Direccion General de Vida Silvestre, SEMARNAP (1996), we are thankful for their expediency and especially their trust in giving us the opportunity to work with two endangered Mexican endemic species. Comments by Noel Snyder to an earlier version were very useful. Thanks also to Kelly Hogan, Gerald D. Lindsey, Claudia Macias, Michael Schindlinger, Elizabeth Stone and 2 anonymous reviewers for suggestions which improved our manuscript.

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