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TECHNICAL REPORT

A Technique for Obtaining Early Life History Data in Pouched Marsupials

Miles Roberts and Frank Kohn

Department of Zoological Research, National Zoological Park, Washington, DC

Early developmental and demographic events in many marsupials occur in the mother's pouch out of animal managers' sight. Because pouch examination is perceived as being stressful both to animal and handler, the pouch has proven to be a formidable physical and psychological barrier to the study of early life history events in marsupials in zoos. Demographic and developmental data such as litter size at birth, secondary sex ratios, infant mortality rates, and early rates of growth and development, normally considered essential information for the development of breeding programs, are therefore essentially unavailable for most zoo populations of marsupials. Here we describe a technique for the safe and non-stressful examination of the pouches of small (<2 kg) marsupials that enables the capture of such data. The technique, involving the use of transparent plastic tubes of slightly different diameters for restraining the animals and an otoscope for examining the pouch and its contents, has been very successfully applied to life history studies of four species at the National Zoological Park: *Pseudocheirus peregrinus*, *Petaurus breviceps*, *Philander opossum*, and *Chironectes minimus*.

Key words: pouch examination, restraint, management

INTRODUCTION

Obtaining accurate data on the early life history of marsupials can be problematical. Most species give birth cryptically and many have pouches in which infants develop, out of sight of the investigator, for periods of a few weeks to many months. Under these circumstances, it can be difficult to accurately determine birth dates, infant mortality and growth rates, birth litter sizes, secondary sex ratios, and other demographic data. While this information is routinely collected in intensive studies of

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Address reprint requests to Miles Roberts, Department of Zoological Research, National Zoological Park, Washington, DC 20008.

marsupial growth and development [see Eisenberg, 1981; Archer, 1982; Smith and Hume, 1984; Zullinger et al., 1984 for examples of such studies], it is not generally obtained under standard zoo management conditions. While marsupial births may be observed in zoos on very rare occasions, it is much more common to first notice young at some indeterminable time after birth as a bulge in the mother's pouch or as a pouch emergent.

The interval between birth and attainment of noticeable pouch size and/or pouch emergence can be quite variable [Cockburn and Lee, 1985]. Developmental rates of pouch young are obviously different among species, but they can also be quite different within species and between litters of the same individual [e.g., Heinsohn, 1966; Stodart, 1977; Smith, 1979; Serena and Soderquist, 1988; Roberts et al., 1990]. A "bulge in the pouch" or "first pouch emergence" is a completely subjective notation dependent on the observer's schedule, his/her observational skills, familiarity with the species and the individual animals, growth rates of the animals, and so forth. At best, even when made under a rigorous observational regime and referenced to accurate published life history data on the appropriate species, these are crude life history notations of questionable statistical value. In the zoo context, this shortfall in neonatal/maternal demographic and developmental data can lead to significant omissions in the design of well-rounded marsupial husbandry and population management programs.

Obtaining the necessary and reliable information generally requires careful examination of the condition and contents of the pouch, a task zoo managers frequently approach with some trepidation. Pouch checking requires capture, restraint, and manipulation of an animal in a way that is safe for it and the handler but effective enough to secure the necessary data. Marsupials are variable enough in size, temperament, and response to handling that it is not always immediately obvious how safe manipulations can be accomplished.

A review of the relevant literature suggests that only a small fraction of marsupial growth and development studies actually describe pouch checking techniques with enough clarity and detail to be replicated by the reader. A large majority of these describe techniques appropriate only for macropods. Most of the pouch checking techniques described for other marsupials (i.e., non-macropods) employ some form of anesthesia, an admittedly risky procedure, but some describe training animals to tolerate physical examination, including pouch checking [Fleay, 1961; Fritz, 1971; Mullett, et al., 1988; Conway, 1988]. The literature suggests that pouch checking can frequently be stressful and dangerous to animals [Shield and Woolley, 1961; Sadleir, 1963; Cisar, 1969; George, 1982; Presidente, 1982; Richardson and Cullen, 1983; Thomason and Russell, 1986; Tribe and Middleton, 1988] or handlers [Calaby and Poole, 1971; Finnie, 1978] and can have detrimental effects on offspring survivorship [Marlow, 1961; Stodart, 1966, 1977; Smith, 1979; Atherton and Haffenden, 1982; Lyne, 1982; Fleming and Frey, 1984]. Clearly, simple and safe techniques still need to be developed.

Here we describe a pouch examination technique for small marsupials (i.e., about 2 kg or smaller) that has proven to be safe and effective for the four species on which it has been applied in our laboratory between 1986 and 1990: common ringtail possum (*Pseudocheirus peregrinus*), sugar glider (*Petaurus breviceps*), water opossum (*Chironectes minimus*), and four-eyed opossum (*Philander opossum*).

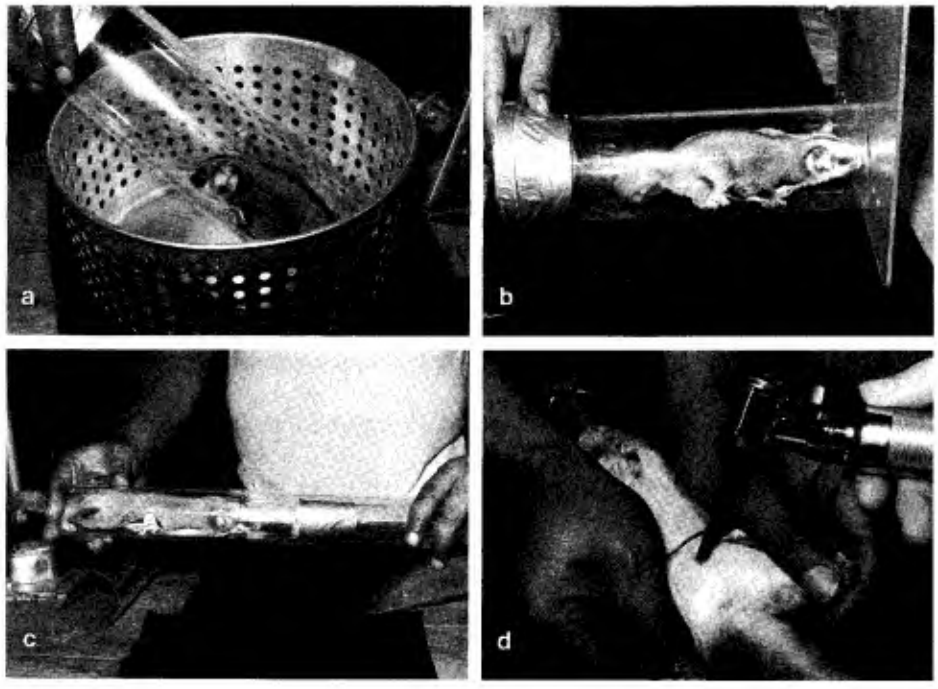

Fig. 1. **a:** Sugar glider transferring from weigh basket to clear plastic tube. **b:** Once in tube animal is calm and is allowed to explore her surroundings. **c:** Plunger tube is introduced. Sugar glider backs up into handler's grasp. **d:** While one handler restrains hind legs lightly, second handler inserts otoscope into pouch. Note relative sizes of pouch opening, speculum, and fingers.

METHODS AND RESULTS

Our technique, a variation on those described previously be Mylrea and Abbrecht [1967] and Amir et al. [1981] for laboratory mice, is designed to minimize handling to reduce the chance of injury to animal and handler. The procedure is performed during the "sleep" period of the animal's day, when it is subdued, more easily accessible, and less excitable. The animal is transferred from a nestbox or small transfer crate, in which it is weighed, into a clear Plexiglas tube of a diameter only slightly greater than that of the animal (tubes are available in various lengths and diameters from Read Plastics, 12331 Wilkins Ave., Rockville, MD 20852) to prevent the animal from turning around in the tube (Fig. 1a,b). A second tube, of slightly smaller diameter than the first, is sealed at one end with fabric or tape to make a plunger. When previously inserted into one end of the larger tube, the plunger blocks the animal's escape. Once the animal is in the tube, it can easily be given a visual examination. Gentle pressure on the plunger is used to manipulate the animal's position in the tube (Fig. 1c). With proper manipulation, the hindquarters and lower abdomen can be pulled from the tube and, with the animal on its back and the hindquarters gently restrained by one person, a second person can examine the pouch. Generally animals are calm in the tube, but a dark cloth draped over the apparatus has an additional calming effect.

The interior of the pouch is examined with an otoscope (Fig. 1d) fitted with one of the several interchangeable conical fittings (specula) normally used for human ear examination (otoscopes are available from most medical or veterinary supply houses). Choice of speculum is determined by the size of the animal. The pouch sphincter is opened by gentle manipulation and insertion of the speculum tip, which has first been disinfected and warmed to room temperature. The otoscope light fully illuminates the pouch interior permitting a clear view of pouch structure and any pouch young. The known diameter of the speculum tip can be used as a measure by which dimensions of the pouch young are taken (e.g., crown-rump length, total length, etc.). When pouch examination is completed, the animal is released directly into its enclosure or nest box simply by removing the plunger and allowing the animal to exit the tube.

The advantages of this pouch checking technique are the following:

1. It requires a minimum of "hands on" restraint thereby reducing the risk of injury to animal or handler.
2. Once the handler learns the procedure, it is quick (about 10 minutes) and the animal remains calm in the device.
3. The otoscope offers a clear and more detailed view of the interior of the pouch than one can get with the naked eye. With an appropriate adapter, photographs and video can be taken directly through the otoscope.
4. Measurements and developmental landmarks of the pouch young can be taken without removing the infants from the teat or pouch, itself a risky practice.

Pouches are checked on a weekly basis during growth and development studies. Animals adjust rapidly to the procedure on this schedule. Of course, some are more tractable than others. We recommend that initially the animals be allowed to examine the tube and to enter it unrestrained. Gradual introduction of the plunger and slowly increasing the animal's time in the tube before even attempting the procedure helps acclimatization immeasurably.

We have no evidence that this procedure has any detrimental effects on adults or offspring. For example, the reproductive rates (number of offspring per reproductive age female per unit time) between our colony of sugar gliders subjected to pouch examination was virtually identical with that of the zoo's exhibit colony in which pouches were not examined. The mortality rate of pouch young in our sugar glider colony was approximately 22% (5 of 22 born), well within the minimum pouch mortality rate of 6–44% reported by Suckling [1984] for a wild population. No other data on pouch mortality of captive sugar glider populations are available with the exception of Smith's [1979] report that "the deaths of many pouch young were probably caused by attacks from adults in their family group when the young were returned to the group after being measured." We did not observe this to occur in any species on which we applied the technique.

DISCUSSION

In zoos, the early developmental and demographic history of pouched marsupials is generally ignored because events occur in the mother's pouch out of the animal manager's view. For most people, the setpoint from which they measure developmental and demographic trends is when they first see either a bulge in the

pouch or first pouch emergence. In many marsupials, first pouch emergence is roughly the onset, but can be as late as the end, of weaning [Tyndale-Biscoe, 1973]. As in placentals, marsupial preweaning is a time of high infant mortality, sex ratio adjustment, high energetic demand on the mother, and the onset of differential growth rates (e.g., between sexes). It has been our experience that knowing what transpires in the preweaning period can be crucial because it is the time in which management intervention can most significantly influence near- and long-term demographic events, for better or worse.

The paucity of early marsupial life history information in zoos may result from the manager's perception that pouch checking is stressful to animal and handler. We recommend the technique described here because we have experienced no apparent negative effects on mothers or young. While we have had excellent results with the otoscope, however, other optical devices, such as boroscopes and fiberscopes, may be just as effective.

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