SHORT NOTES

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FAECAL COLLECTOR FOR FIELD STUDIES OF DIGESTIVE RESPONSES IN FOREST TORTOISES

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Over the years, many studies have been conducted on passage of digesta through the vertebrate gut, particularly in species considered to be important endozoochorous agents, i.e. species that disperse seeds in their faeces (Karasov *et al.*, 1986; Hume, Morgan & Kenagy, 1993; Martínez Del Rio & Restrepo, 1993). In endozoochorous seed dispersal, seed survival depends largely on how well the seeds are adapted to pass safely through the digestive tract. Gut passage is a delicate but necessary stage for seeds that are dispersed endozoochorously (Stevens & Hume, 1995). Many authors consider zoochory to be a means for seeds to (1) be deposited in microsites with physical and biological characteristics that increase the probability of successful germination and establishment (Augspurger, 1984; Howe, 1993*a,b*); (2) escape from natural predators (Schupp, 1988; Hulme, 1998; Hulme, Lins E Silva & Sousa, 2000); and (3) reduce intraspecific competition (Loiselle, 1990; Notman, Gorchov & Cornejo, 1996). In some plant species, seed passage through a dispersal agent's gut significantly increases the proportion of seeds germinating and/or their speed of germination (in chelonians: Braun & Brooks, 1987, Cobo & Andreu, 1988; in lizards: Willson *et al.*, 1996; in birds: e.g. Izhaki & Safriel, 1990; in mammals: e.g. Julliot & Sabatier, 1993).

The great majority of studies on digestive processes have been conducted on animals in captivity, under controlled conditions. These studies have shown that digestive processes, particularly digesta transit rate, depend on a variety of factors, such as temperature (Parmenter, 1981, Spencer, Thompson & Hume, 1998) and the composition and volume of digesta (Bjorndal, 1989; Clench & Mathias, 1992; Brand, Lanyon & Limpus, 1999). The combined effects of these factors make the understanding of how they affect the fate of seeds a complex matter (Bjorndal, 1991).

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Most studies on endozoochory have two main objectives: (1) to determine the effects of gut passage on seed germination; and (2) to appreciate effects of scat deposit site on seedling establishment. To conduct such studies, it is necessary to collect animal faeces. How this is achieved mostly depends on the habitat of the animal that is studied. While it is relatively easy to collect faeces of animals living in open habitats, it is much more difficult in dense vegetation such as in tropical rain forests. In the latter, the success of faeces collection will depend largely on the type of animal studied. For instance, in some primate species it is relatively easy to detect droppings falling down from the canopy, especially when the animals defecate daily at fixed times and locations (Julliot, 1996). In contrast, it is much more difficult to find scats of more inconspicuous animals, such as tortoises, that live in dense vegetation at ground level.

The South American yellow-footed tortoise (*Chelonoidis denticulata*) is a typical species of South American tropical rain forests. It has a wide geographic range throughout northern South America, and is absent from only a few areas (e.g. Northern Colombia, Paraguay). On average, the linear length of the shell varies between 250 mm and 330 mm, and it weighs between 3.5 kg and 5.0 kg (Métrailler & Le Gratiet, 1996). During 15 months of field work at the Scientific Station of Nouragues (C.N.R.S. UPS 656; 4°05'N, 52°40'W) in French Guiana, I found no tortoise scats in the field, even when using a spool-and-line method to study movement patterns. Therefore, to obtain the faeces required for the study of their natural diet, tortoises were captured and confined individually in closed, plastic containers until they defecated (generally within 30 min), after which they were released. That study showed that *Chelonoidis denticulata* is a generalist, opportunistic feeder that feeds mainly on a variety of fruits. Thus, *C. denticulata* might be an important endozoochorous agent within neotropical rain forests.

For various reasons, "stress faeces" collected as described above cannot be used to determine parameters such as mean retention time, defecation rhythm and, especially, effects of gut passage on seed germination. For example, it is usually not known when the seeds found in such faeces were ingested, and seeds may not have undergone the whole action of natural gut passage. Thus, a faecal collector would be a useful device to easily obtain naturally deposited faeces in the field.

Bjorndal (1991), and Avery *et al.* (1993) used a semi-permanent device to collect all excreta voided by *Trachemys scripta*. Their apparatus consisted of a modified tube connector that was placed over the turtle's tail and either glued to the turtle's skin at the base of the tail with a silicone sealant, or held around the tail with a wire fitted around the tube and through two holes drilled in the carapace. A large latex finger cot was attached to collect all excreta, while spaces between the tubing and the skin and the shell of the turtle were sealed with silicone. In studies on tortoises, Bjorndal (1987, 1989) and Meienberger, Wallis & Nagy (1993) used cloth bags

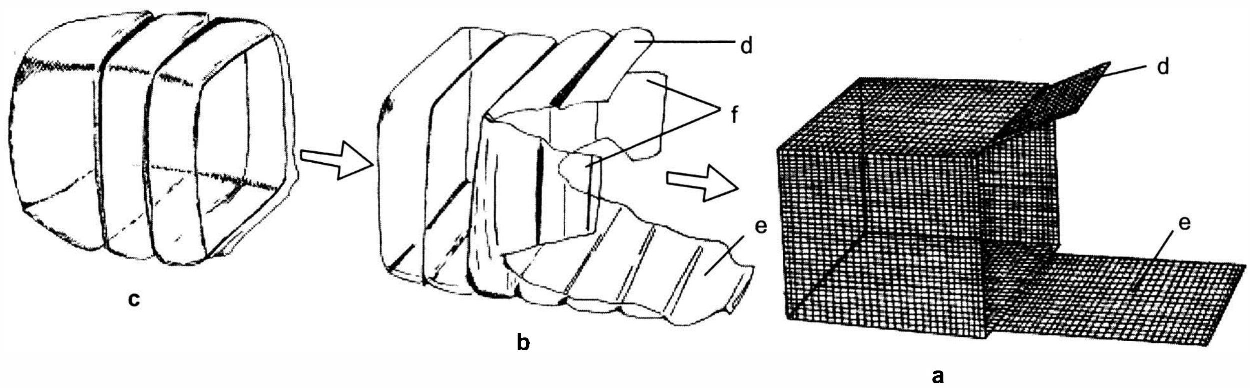

FIG. 1. View showing each part of the faecal collector separately: the mesh wire framework (a) with its two flaps, the plastic base (b) and the plastic cap (c).

that were attached to the animals by threads passed through holes drilled in the rear of the carapace. The animals were housed in individual pens where the ground was cleared of all objects that might tear the cloth bags. Other experiments with faecal collectors were conducted on animals housed in individual cages (Barboza, 1995; Hailey, 1998). All of these devices were designed to be used on animals maintained in captivity, i.e. in a simplified environment where most conditions were controlled (Hamilton & Coe, 1982). All the latter techniques gave good results in their respective circumstances but were basically unsuitable for use in studies on free-ranging animals in complex environments. To enable the study of digestive processes in free-ranging tortoises, I designed a faecal collector that could be adapted to fit tortoises and that would withstand their normal negotiation of obstacles in their natural environment.

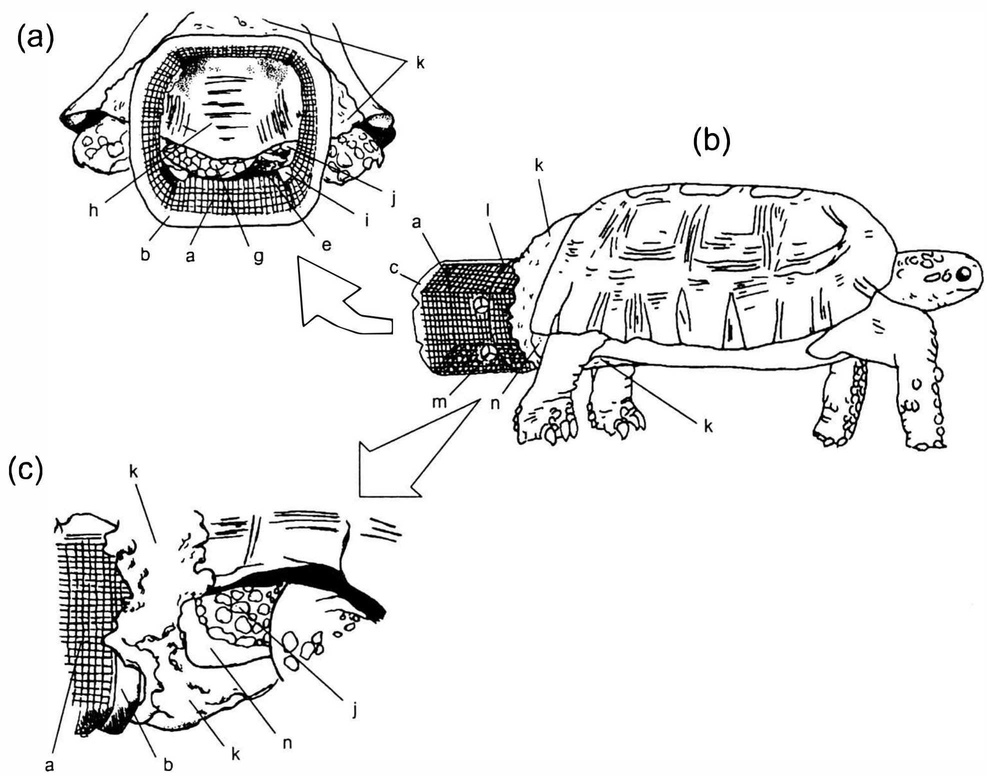

FIG. 2. (a) Back view showing the inside of the faecal collector without the removable cap. The tail is maintained naturally curved. The space between the shell and the plastron flap allowed the stretching of the tail along the plastron flap. (b) female equipped with a faecal collector. This device consisted of a wire-mesh framework attached to the rear of the carapace, with two plastic parts fitted around it and maintained with two bolts. (c) Details at the level of the hind foot. The framework and the plastic base were attached to the rear of the carapace with the Sintofer filler. Open spaces around the thighs were filled-in with a silicone sealant. No faecal matter could seep out. Key: a, mesh wire framework; b, plastic base; c, plastic cap; d, shell flap; e, plastron flap; f, lateral flaps; g, tail; h, supracaudal scute; i, space closed by the plastic base; j, thigh; k, Sintofer filler; l, plastic base limit under which the cap fitted into the base; m, bolt; n, silicone sealant (Drawings: S. Jouard).

The faecal collector consisted of three different parts: a wire-mesh framework and a soft, plastic casing in two parts (Fig. 1). The mesh framework formed a rectangular cage (with the lateral edges tied together with galvanized wire), 65 mm x 65 mm in cross section, 70 mm long, and open at both ends. Two flaps at one end were fitted tightly around the carapace and the plastron. The shape of the two flaps could be modified to fit exactly the shape of the shell, which varies between individuals. The flaps were 50 mm wide and either 40 mm long (carapace flap) or 80 mm long (plastron flap). The greater length of the plastron flap accommodated the more anterior position of the edge of the plastron and the concavity of the male plastron. The supracaudal scute of males is inwardly curved, reducing the angle of axial stretching of the tail; this required the plastron flap to be curved slightly, lowering the device a few centimetres towards the ground. As the female plastron is straight, the device was mounted level and continuous with the plastron (Fig. 2b).

The plastic casing was made from a 1.5-litre plastic bottle, square in cross section, in which drinking water was sold. The bottle was slightly larger in cross section than the mesh framework, and so fitted neatly around it. The bottle collected liquid faecal matter that might otherwise seep through the mesh, and also protected the collected faecal matter from rainfall. The base of one bottle was used as a removable cap that fitted over the bottomless, lower part of a second bottle (Fig. 1). The plastic casing had two flaps that were slightly shorter than those of the mesh framework (Fig. 1); the plastic flaps were drilled and attached to their corresponding mesh flaps with wire. The plastic casing was trimmed to fit the carapace closely, filling any gaps at the edges of the mesh frame. The base of the device had to stop at the level of the marginate scutes, in order not to hamper the locomotion of the tortoise (Fig. 2c). Thus, profiling the flaps demanded painstaking attention to detail. Once the plastic base was shaped, the device was attached to both shell and plastron with a two-component filler designed for repairing coachwork (Sintofer standard polyester filler, Sinto, Aubagne, France). Once dried, the filler stuck very well to the rough surface of the carapace, while both the casing and the frame were embedded tightly in the filler.

Finally, to avoid loss of faecal matter around the hind feet, open spaces were filled-in with a waterproof silicone sealant (Rubson silicone made by Henkel, France SA; Fig. 2c). Once dried, this material had to adhere well to the animal's skin, otherwise it might have fallen off and left a gap. Such an opening would have remained small while the animal was walking and in most other positions. Only if the tortoise retracted wholly into its shell (e.g. if danger threatened) would quite a large space have appeared; in the unlikely event of the collector being full, leakage might then occur. The removable cap facilitated quick retrieval of collected faeces. The cap was held in place by two parallel, transverse bolts that prevented it from revolving around its axis and thus prevented leakage (Fig. 2b).

In tortoises, the tail is usually curled in the space between the carapace and the plastron (Fig. 2a) and is only extended when the animal defecates and during mating. The device was designed so that it would not disturb this extension. All wire-mesh edges that might have come in contact with the tortoise's skin were curved and coated in silicone to prevent injuries. After use, it was relatively easy to remove the device with a hammer and a chisel, without injuring the animal. Usually, the Sintofer filler could be removed all in one piece, leaving the carapace clean. A health inspection of the tortoises at the end of the experiment revealed no injuries caused by the device.

For use on sub-adult tortoises a smaller version of the faecal collector was made from a 0.75-litre plastic bottle and a wire mesh frame 42 mm long and 62.5 mm on each side. The two mesh flaps were 45 mm wide and 20 mm and 40 mm in length for the carapace flap and plastron flap, respectively.

Ten adult *C. denticulata* were fitted with the faecal collector, as described. The mean mass (±SD) of the collectors was 302±34 g and the mean volume was 309±53 ml, representing 8.1±1.7% of the body mass and 7.5±1.1% of the volume, respectively, of the equipped tortoises. Six faecal collectors were equipped with transmitters for radio-tracking. The mean mass (±SD) of the transmitters was approximately 85±5 g and the whole equipment (collector + transmitter) weighed 401±42 g (n=6), which was 9.9±1.5% of the body mass. The smaller collector fitted to a sub-adult had a mass of 175 g and volume of 164 ml, corresponding to 8.0% of the body mass and 9.7% of the volume of the tortoise, respectively.

During the present study, the Sintofer filler used to attach the collector to the tortoise's back was resistant to moisture and all other factors impinging on the device. Unlike previously described devices, this faecal collector did not suffer from forces of tearing or compression and none was torn away from the free-ranging animals over periods of 45 days.

As the plastic bottles were transparent, the presence of faeces could be detected easily, albeit with the aid of a flashlight when the device was dirty. Thus, tortoises could be checked several times a day to estimate defecation rhythm and digesta transit time, but with minimal disturbance. Faeces were removed, when detected, by unscrewing the bolts and removing the cap. In general, the retrieval of faeces took less than 10 min and could usually be done at the site where the tortoise was found. The time elapsed between consecutive defecations ranged from one day to more than 38 days and depended greatly on the activity level of the tortoise. Consequently, any stress induced by the retrieval of faeces was infrequent.

Once fitted, the collector (with its additional transmitter) did not protrude above or to the sides of the carapace. Consequently, most normal movements of the animals were not hindered. However, the additional length at the back of the shell (73±13 mm, n=10) might have hampered the animal when turning in very restricted places, such as tree-fall gaps, which are important as refuges. Moskovits (1985) showed that gaps represented half of all shelters used by *Chelonoidis denticulata*, whereas at Nouragues they represented on average 44% of the shelters used (Josseaume, 2002). As tree-fall gaps constituted 62% of all shelters used by tortoises equipped with the faecal collector in this study, it appears that the collector did not prevent use of this important refuge. In terms of home range, the great individual variability observed in many tortoise species (e.g. Stickel, 1950; Kiester, Schwartz & Schwartz, 1982), including *Chelonoidis denticulata* (Moskovits, 1985; Josseaume, 2002), demands large sample sizes to compare tortoises with and without a faecal collector. At Nouragues, several individuals were followed using a spool-and-line method, or by radio-tracking, to study their displacement patterns. The mean home range (± SD) of tortoises without a faecal collector, determined by the minimum convex polygon method, was 5.4 ±3.0 ha (n=7; minimum 2.3 ha; maximum 8.9 ha), whereas that of free-ranging individuals equipped with a faecal collector was 3.2± 2.5 ha (n=6; min: 0.9 ha; max: 7.7 ha). The difference between the two samples was not statistically significant (Mann-Whitney U-test: U=32, P=0.116). Foraging patterns and feeding rates of animals with and without faecal collectors appeared similar during direct observation of behaviour (Josseaume, 2002).

The carapace of tortoises constitutes a good support for devices such as the faecal collector described here. In general, these animals are both easy to handle and very sturdy. Therefore, a small additional mass does not affect their locomotion capacity (Zani & Claussen, 1994, 1995). For the researcher, this faecal collector is simple to set up and use. It is adjustable for the sex and the dimensions of the animal. The materials are easy to manipulate; the wire mesh is flexible, yet strong enough to resist both lateral and axial pressures resulting from the movements of a tortoise in cluttered parts of its habitat, such as tree-fall gaps.

In a study of the role of *Chelonoidis denticulata* as a seed disperser, the faecal collector permitted collection of seeds that had undergone the whole action of natural gut passage, in dense forest understory where it was practically impossible to find scats. This contributed greatly to the study of gut digesta passage and defecation patterns under natural conditions. The mean retention time in the field could be determined by feeding the tortoises with exogenous markers (i.e. non-indigenous seeds) just before they were released, while data on the daily displacements of the species could help in assessing the dispersal of tortoise-ingested seeds. Effects on ingested seeds could be studied with germination tests. Also, scat deposit sites, which may be important in the study of directional seed dispersal (Howe & Smallwood, 1982), could be determined. This could be realized in an indirect way by combining the use of faecal collectors and radio-tracking.

In conclusion, the present faecal collector offers many new possibilities for the study of natural digestive responses in tortoises living in inaccessible habitats that are usually not amenable to this type of research.

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REFERENCES

Augspurger, C. K. (1984). Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* **65**, 1705-1712.

Avery, H. W., Spotila, J. R., Congdon, J. D., Fischer, R. U. J., Standora, E. A. & Avery, S. B. (1993). Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. *Physiological Zoology* **66**, 902-925.

Barboza, P. S. (1995). Digesta passage and functional anatomy of the digestive tract in the desert tortoise (*Xerobates agassizii*). *Journal of Comparative Physiology B* **165**, 193-202.

Bjorndal, K. A. (1987). Digestive efficiency in a temperate herbivorous reptile, *Gopherus polyphemus*. *Copeia* **3**, 714-720.

Bjorndal, K. A. (1989). Flexibility of digestive responses in two generalist herbivores, the tortoises *Geochelone carbonaria* and *Geochelone denticulata*. *Oecologia* **78**, 317-321.

Bjorndal, K. A. (1991). Diet mixing: nonadditive interactions of diet items in an omnivorous freshwater Turtle. *Ecology* **72**, 1234-1241.

Brand, S. J., Lanyon, J. M. & Limpus, C. J. (1999). Digesta composition and retention times in wild immature green turtles, *Chelonia mydas*: a preliminary investigation. *Marine and Freshwater Research* **50**, 145-147.

Braun, J. & Brooks, G. R. J. (1987). Box turtles (*Terrapene carolina*) as potential agents for seed dispersal. *American Midland Naturalist* **117**, 312-318.

Clench, M. H. & Mathias, J. R. (1992). Intestinal transit: how can it be delayed long enough for birds to act as long-distance dispersal agents? *The Auk* **109**, 933-936.

Cobo, M. & Andreu, A. C. (1988). Seed consumption and dispersal by the spur-thighed tortoise *Testudo graeca*. *Oikos* **51**, 267-273.

Hailey, A. (1998). The specific dynamic action of the omnivorous Tortoise *Kinixys spekii* in relation to diet, feeding pattern, and gut passage. *Physiological Zoology* **71**, 57-66.

Hamilton, J. & Coe, M. (1982). Feeding, digestion and assimilation of a population of giant tortoises (*Geochelone gigantea* (Schweigger)) on Aldabra atoll. *Journal of Arid Environments* **5**, 127-144.

Howe, H. F. (1993*a*). Aspects of variation in a neotropical seed dispersal system. In: *Frugivory and Seed Dispersal: Ecological and Evolutionary Aspects*, 149-162. Vol. 107/108. Fleming, T. H. and Estrada, A. (Eds): Vegetatio.

Howe, H. F. (1993*b*). Specialized and generalized dispersal systems: where does 'the paradigm' stand? In: *Frugivory and Seed Dispersal: Ecological and Evolutionary Aspects*, 3-13. Vol. 107/108. Fleming, T. H. and Estrada, A. (Eds): Vegetatio.

Howe, H. F. & Smallwood, J. (1982). Ecology of dispersal. *Annual Review of Ecology & Systematics* **13**, 201-228.

Hulme, P. E. (1998). Post-dispersal seed predation consequences for plant demography and evolution. *Perspectives in Plant Ecology, Evolution and Systematics* **1**, 32-46.

Hulme, P. E., Lins E Silva, A. C. B. & Sousa, Z. S. (2000). An assessment of the relative importance of different livestock in the dispersal of an invasive alien legume (*Prosopis juliflora*) in Northeast Brazil. In: *3rd International Symposium-Workshop on Frugivores and Seed Dispersal, Biodiversity and Conservation Perspectives. Program and Abstracts*, 59. Hotel Fazenda Fonte Colina Verde , São Paulo , SP - Brasil: Universidade Estadual de Campinas, Universidade Estadual Paulista.

Hume, I. D., Morgan, K. R. & Kenagy, G. J. (1993). Digesta retention and digestive performance in sciurid and microtine rodents: effects of hindgut morphology and body size. *Physiological Zoology* **66**, 396-411.

Izhaki, I. & Safriel, U. N. (1990). The effect of some Mediterranean scrubland frugivores upon germination patterns. *Journal of Ecology* **78**, 56-65.

Josseaume, B. (2002). Rôle des Chéloniens dans la régénération des écosystèmes forestiers tropicaux - Exemple de la Tortue denticulée (*Chelonoidis denticulata*) en Guyane française. Thèse de doctorat. Université Paris VI. 428pp.

Julliot, C. (1996). Seed dispersal by red howling monkeys (*Alouatta seniculus*) in the tropical rain forest of French Guiana. *International Journal of Primatology* **17**, 239-258.

Julliot, C. & Sabatier, D. (1993). Diet of the red howler monkey (*Alouatta seniculus*) in French Guiana. *International Journal of Primatology* **14**, 527-550.

Karasov, W. H., Petrossian, E., Rosenberg, L. & Diamond, J. (1986). How do food passage rate and assimilation differ between herbivorous lizards and nonruminant mammals? *Journal of Comparative Physiology B* **156**, 599-609.

Kiester, A. R., Schwartz, C. W. & Schwartz, E. R. (1982). Promotion of gene flow by transient individuals in an otherwise sedentary population of box turtles (*Terrapene carolina triunguis*). *Evolution* **36**, 617-619.

Loiselle, B. A. (1990). Seeds in droppings of tropical fruit-eating birds: importance of considering seed composition. *Oecologia* **82**, 494-500.

Martínez Del Rio, C. M. & Restrepo, C. (1993). Ecological and behavioral consequences of digestion in frugivorous animals. In: *Frugivory and Seed Dispersal: Ecological and Evolutionary Aspects*, 205-216. Vol. 107/108. Fleming, T. H. and Estrada, A. (Eds): Vegetatio.

Meienberger, C., Wallis, I. R. & Nagy, K. A. (1993). Food intake rate and body mass influence transit time and digestibility in the desert tortoise (*Xerobates agassizii*). *Physiological Zoology* **66**, 847-862.

Métrailler, S. & Le Gratiet, G. (1996). *Tortues continentales de Guyane française*. Bramois: P.M.S. Switzerland.

Moskovits, D. K. (1985). The behavior and ecology of the two Amazonian tortoises, *Geochelone carbonaria* and *Geochelone denticulata*, in Northwestern Brasil. PhD. University of Chigago 328 pp.

Notman, E., Gorchov, D. L. & Cornejo, F. (1996). Effect of distance, aggregation, and habitat on levels of seed predation for two mammal-dispersed neotropical rain forest tree species. *Oecologia* **106**, 221-227.

Parmenter, R. R. (1981). Digestive turnover rates in freshwater Turtles: the influence of temperature and body size. *Comparative Biochemistry and Physiology* **70A**, 235-238.

Schupp, E. W. (1988). Seed and early seedling predation in the forest understory and in treefall gaps. *Oikos* **51**, 71-78.

Spencer, R.-J., Thompson, M. B. & Hume, I. D. (1998). The diet and digestive energetics of an Australian short-necked turtle, *Emydura macquarii*. *Comparative Biochemistry and Physiology* **121A**, 341-349.

Stevens, C. E. & Hume, I. D. (1995). *Comparative physiology of the vertebrate digestive system.* Cambridge: Cambridge University Press.

Stickel, L. F. (1950). Populations and home range relationships of the box turtle, *Terrapene c. carolina* (Linnaeus). *Ecological Monographs* **20**, 351-378.

Willson, M. F., Sabag, C., Figueroa, J., Armesto, J. J. & Caviedes, M. (1996). Seed dispersal by lizards in Chilean rainforest. *Revista Chilena de Historia Natural* **69**, 339-342.

Zani, P. A. & Claussen, D. L. (1994). Voluntary and forced terrestrial locomotion in juvenile and adult painted turtles, *Chrysemys picta*. *Copeia* **1994**, 466-471.

Zani, P. A. & Claussen, D. L. (1995). Effects of extrinsic load on locomotion in painted turtles (*Chrysemys picta*). *Copeia* **1995**, 735-738.

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