The gut passage time in Myanmar Star Tortoise (*Geochelone platynota*) and its role for seed dispersal

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Abstract

Seed dispersal is one of the most important plant-animal mutualisms. Seed dispersal by mammals and birds is well studied but dispersal of seeds by reptiles (saurochory) is poorly studied. The role of Myanmar Star tortoises as seed dispersers is studied by gut passage time and germination experiment. Study of gut passage time of Myanmar Star Tortoises, *Geochelone platynota* was conducted from 9thDecember 2018 to 5thJanuary 2019 in Minsontaung Wildlife Sanctuary (21°24'-21°27'N and 95°46'-95°49'E), Ngatogyi Township, Mandalay Region. A total of 600 seeds including papaya, jackal jujube and guava were fed to the four tortoise groups in captive condition and 78.8% of these were recovered. The duration of first seeds appeared in 3 days after ingestion and the longest seed was observed after 23 days. Germination experiments on treatment(seed ingested by tortoises) and control (seed not ingested by tortoises)were conducted and compared by groups from April, 2018 to June, 2019. Seeds were planted in soil-filled trays to determine the effect of digestive tract on germination. Germination percentage (percentage of seeds that germinated during the experiment) and germination rate (numbers of days from planting to seedling emergence) of seeds from two groups were compared to evaluate the effect of tortoises on germination. The tortoises dispersed *Ziziphus oenoplia* in the wild and gut passage significantly enhanced germination percentage of *Ziziphus oenoplia*. This study shows the important of tortoises in the ecosystem.

Keywords: plant-animal mutualism; saurochory; gut passage time; germination; *Ziziphus oenoplia*

Introduction

The Myanmar Star Tortoise, *Geocheloneplatynota* (Blyth, 1863) (Family Testudinidae) is a medium- sizetortoise(carapace length to 30cm)endemic to the dry zone of central Myanmar. Of all tortoises characterized by the highly distinctive 'star' or 'radiating' patterns on their carapace, the Myanmar Star Tortoise is perhaps the rarest and most beautiful. Carapace is dark brown or black with six or fewer radiating stripes extending from the yellow areola of each vertebral and pleural. Two yellow stripes form a "v" shape pattern on each marginal. Plastron is yellow and black blotches. *G. platynota* was designated a member of "extinction row" along with other high-rick *cheolonian* taxa (Buhlmann*etal*., 2002), listed among the 25 most endangered chelonians in the world (Rhodin*etal*, 2011), and assessed as Critically Endangered by the IUCN (IUCN 2014).

Plants are sessile for the most of their life cycle, but they do move across the landscape (Shea 2007). For this, plants employ two main strategies: pollen and seed dispersal. Seed dispersal is the link between the end of the reproduction and the establishment of new vegetative growth units (Schupp 1993; Schupp et al 2010; Wang & Smith 2002). In many areas in the tropics up to 80-90% of plants depend on animals ingesting their seeds for dispersal (endozoochory, Howe& Smallwood 1982).

Animal-mediated seed dispersal is an important ecosystem function that determines the structuring of plant populations and biodiversity, and has profound ecological and evolutionary implications (Howe& Smallwood 1982; Stoner & Henry 2008). Endozoochory, which is the dispersal of seeds ingested by animals, is the most common form of animal-

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mediated seed dispersal, with as many as 75% of plants in tropical forests depending on animals to disperse their seeds (Howe & Smallwood 1982).

A major goal of seed dispersal ecology is to identify not only which seeds are being dispersed by what frugivores, but the proportion of seeds dispersed by a given animal that will germinate, and ultimately grow into a mature plants – the so-called seed dispersal efficiency (SDE) concept (Schupp 1993; Schuup et al 2010).

Zoochory is one of the several methods of plant seed dispersal (Corlett 1995; Wunderle 1997; Corlett 1998). Seed dispersal is an ecological process that enables a plant to disperse from the parent plant to places that favour their establishment. The spreading of seeds away from the parent plant may lead to new sprouting grounds. The falling of seeds beneath parent plants may result in seedling facing competition, risks of inbreeding and being infected with diseases (Fragoso et al., 2003).

Most studies of zoochory and seed dispersal effectiveness have focused on birds and mammals which are often dispersers of prodigious volumes of seeds (Howe 1990; Jordano 2001), though other taxa including reptiles and fish may also be effective dispersers (Corlett 1998). In terrestrial ecosystems, tortoises contribute to seed dispersal dynamics (Liu et al., 2004; Birkhead et al., 2005; Jerozolimski et al.,2009).

Among factors that influence dispersal quality, digesta retention time may be of particular importance since it determines how long seeds are exposed to potentially advantageous and/or damaging digestive processes, and it influences the spatial scale and pattern of seed disposition. Trade-offs in seed dispersal effectiveness may exit among these effects of retention times; for example long retention time may increase the probability of long distance dispersal (Blake et al., 2009).

Gut retention time (GRT; the time seeds are retained in the digestive tract) is one measure of SDE. Retention time in the gut of frugivores may determine the distance of dispersal and the structure of the seed rain shadow. The treatment of seeds in the gut can also affect seed viability (Schuup 1993; Traveset 1998). Moreover, temporal retention of seeds and subsequent deposition away from the mother plants may aid seeds in escaping predation, affect their germination and determine the outcome of seedlings as adult recruits (Connel 1971; Guzman & Stevenson 2011; Janzen 1970).

Environmental change whether natural or anthropogenic, is constantly altering local habitat conditions that influence demographic processes in plants. When previously unsuitable habitat conditions become suitable for establishment, successful colonization depends on dispersal of seeds in to the altered habitat (Cain *et al*., 2000).

The overreaching goal of this study is to better understand the role of *Geochelone platynota*as agent of seed dispersal in dry zone forests of central Myanmar.

The present study was carried out the following objectives:

- to estimate the gut passage time of feed (seeds) in Myanmar Star Tortoises kept in captivity

- to determine whether the size of tortoises effect on the gut passage time

- to study the effect of digestive tract of tortoise in seed germination in Minsontaung Wildlife Sanctuary.

Materials and Methods

Study Area

This study was conducted in Minsontaug Wildlife Sanctuary. Minsontaung Wildlife Sanctuary (20˚ 11'N; 94˚ 28'E) is located in Ngatogyi township, Mandalay Division. The sanctuary was established in 1998-99 and comprises 2260 ha.

Methods

Gut Passage Time

Study Periods

This study was carried out from 9th December 2018 to 5th January 2019.

Selection of the Tortoises

Twenty tortoises of various sizes were selected and divided into four groups(Group I to IV) to determine the retention time of food in the digestive tracts of tortoises.

Types of Seeds

Three different types of seeds were used in this study to see the different passage time through the gut. Natural seeds from three types of fruits: jackal jujube(Taw-Zee), papaya and guava were taken and then fed them embedded in papaya pulp.

Feeding and Seed Recovery

To estimate the gut passage time of ingested seeds by the Myanmar Star Tortoises, three types of seeds described above, were given to the tortoises and were later on recovered in their feces (Murphy & Sicilian-Jones 1986, Willson 1989, Rick & Bowmen 1960, Levey & Sargent 2000). Fifty seeds of each type were given to each group. The dungs were collected and washed under the tap water in a fine-mesh sieve to get the ingested seeds. The number of seeds and dates were noted down.

Germination

To determine which species of plants might be dispersed by *Geochelone platynota,* feces were collected directly from tortoises being monitored as part of an on-going reintroduction project at Minsontaung Wildlife Sanctuary (Platt &Platt,2016). Then, seeds from fruits (or foliage) ingested by tortoises were recovered by washing feces in fine-mesh sieve. Seeds from the feces and seeds collected directly from the plants were planted in soil-filled trays to determine of the effects of gut passage on germination rate (number of days from planting to seedling emergence) or germination percentage (percentage of seeds that germinate during experiment).

Statistical Analyses

. All statistical analyses were performed with SPSS version 23.The effect of seed types, the tortoise groups on the first, median and longest appearance times were analyzed and significant effects were investigated with Scheffe.

Results

Total Amount of Seed Recovery

A proportion of 78.8% of the 600 seeds ingested by all tortoise groups was recovered. A total of 78% of papaya seeds, 81.5% of jackal jujuble seeds, and 77% of guava seeds were recovered. The earliest recovery was 3 days and the longest retention was 23 days after ingestion. The first appearance time, the median appearance time, and the longest appearance time of all seed types are described in Table7.

The result of the statistical analyses of variability in the gut passage time between all tortoise groups fed with three types of seeds are described in Table (8,9,10). There was no significant difference in the first and median appearance time of all tortoise groups. But the significant difference was discovered in the longest appearance time (Table 10 and Appendix). Means of sample groups of the first appearance times showed that the first and last groups do not differ significantly each other but the group II and III differ from each other and the other two groups (Fig.3a). Means of sample groups of the median appearance times described that all four groups differ from each other and the gut passage time increases from the first group to the last group in Fig.3b. Means of sample groups of the longest appearance times showed that the first and second groups are different from each other and the other two groups while the group III and IV do not differ from each other (Fig. 3c).

Table (1). The first, median, and longest appearance time (days) of the tortoise groups

Tortoise Group	First Appearance Days			Median Appearance Days			Longest Appearance Days		
	Papaya	**Jackal Jujube**	**Guava**	**Papaya**	**Jackal Jujube**	**Guava**	**Papaya**	**Jackal Jujube**	**Guava**
Group I	3	9	5	11	12	13	21	22	21
Group II	3	7	9	7	17	17	21	23	23
Group III	4	5	6	13	12	18	23	23	23
Group IV	5	6	7	13	16	17	23	23	23

Table (2). The first appearance times of the three seed types analyzed with ANOVA.

First Appearance (days)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.917	3	.972	.188	.902
Within Groups	41.333	8	5.167		
Total	44.250	11			

Table (3). The median appearance times of the three seed types analyzed with ANOVA.
Median Appearance (days)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	17.667	3	5.889	.481	.705
Within Groups	98.000	8	12.250		
Total	115.667	11			

Table (4). The longest appearance times of the three seed types analyzed with ANOVA.

Longest Appearance (days)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5.583	3	1.861	4.467	.040
Within Groups	3.333	8	.417		
Total	8.917	11			

Germination

Ziziphus oenoplia fruits were available from December to February and seeds were recovered from the feces. The mean germination percentage for seeds recovered from the tortoise feces and control seeds collected directly from the plants was 65.0 (±7.07) and 43.33 (±4.71), respectively. The proportion of dead seeds in the feces was significantly less than in controls (Chi-square= 9.74, df=1, P=.002). In recent germination test, seeds of *Ziziphus oenoplia* (Taw-Zee) indicated that passage through the digestive tract changes the germination percentage compared to undigested seeds.

Table (5). Results of germination test of uneaten seeds and seeds from feces for *Ziziphus oenoplia Ziziphus oenoplia*

Germination interval	Treatment one (seeds from feces)	Control one (uneaten seeds)	Treatment two (seeds from feces)	Control two (uneaten seeds)
0-7	0	0	0	0
7-14	9	8	7	5
14-21	10	4	8	4
21-28	2	0	3	5
28-35	0	0	0	0
35-42	0	0	0	0
42-49	0	0	0	0
Total (percent) Germinated	21 (70.0%)	12 (40%)	18 (60%)	14 (46.6%)
No. Seeds Overall	30	30	30	30

Discussion

The number of seeds recovered from the four different groups differed from each other. Rick & Bowman (1960) described that 23.5%, 24.5% and 42% of the ingested seeds were recovered during the three feeding tests on Galapagos tortoises. A total of 78.8% of the total 600 seeds ingested by all tortoise groups were recovered in the present feeding test. The minimum seed recovery of 77.3% was obtained in Group I and the maximum of 81.3% was discovered in Group II.

Wilfredo Falcon, Nancy Bunbury and Dennis M. Hansen (2018) reported that both small and large are capable of retaining small and large seeds in their guts for 2-4 weeks. Various sizes of tortoises used in the present feeding test are able to retain the different seed sizes in their digestive tracts for 3-23 days.

Setlalekgomo andSesinyi (2014) described that the retention time of the serrated tortoises (*Psammobatesoculiferus*) were recorded from three to seven days. Sadeghayobi*et al.*

(2011) found that the digesta retention time in the Galapagos tortoises (*Chelonoidisnigra*) ranged from 6 to 28 days.

Means sample groups of the first appearance time showed that the second and third groups differed from each other but the first and fourth groups did not differ significantly. The median appearance times were different in the four tortoise groups. The median appearance times slightly increased from the first group to the last group. The size of the tortoises increased from the first group to the last group .This explained that the gut passage time was shorter for the small tortoises than the large ones. The metabolic rates for all organisms, including reptiles, are affected by temperature and size are lower for larger body mass (Gillooly*et al.* 2001).The longest appearance time of the third and fourth groups was similar but different from the first group and the second group.

When the relatively small box turtle (*Terrapene Carolina*) was fed fruits of common wild plant species, seed size did not influence GRT, but it did influence the percentage of seeds that passed intact, with larger seeds being more likely to be defecated (Braun & Brooks, 1987). The body mass did not influence the GRT of Galapagos giant tortoises (Sadeghayobi*et al*., 2011). Waibel et al. (2013) described that sub-adult Aldabra giant tortoises had a shorter GRT than adults of fruit seeds. The present study showed that GRT was slightly shorter for the smaller tortoises than the larger ones.

The result of the statistical analyses of variability in the gut passage time between all tortoise groups fed with three types of seeds in Table (8,9,10 and Appendix) showed that there was no significantly difference between the tortoise groups in the first and median appearance times but the longest appearance time was significant between the first group and the third, the first and last group, F (3,8)=4.467, P=0.040. Myanmar star tortoises dispersed *Ziziphus oenoplia* plant species in Minsontaung Wildlife Sanctuary because seeds were found in the feces of tortoises. Seeds from the feces had higher percentage of germination as compared to uneaten seeds in recent germination tests. The germination percentages of uneaten seeds were 40.0 and 46.66 respectively while that of seeds from the feces were 70.0 and 60 (Table. 5).

The study showed that tortoise gut passage enhances seed germination percentage. This agreed with Rick and Bowman (1961), who described that the gut passage of Galapagos tortoises improved the germination of the Galapagos tomato seeds. Liu *et al*.(2004) reported that the gut passage of the Florida box turtle (*Terrapene Carolina bauri*) greatly enhanced the germination percentage and germination rate of Serenoa repens seeds but decreased the germination percentage of *Thrinax morrissii* and *Byrsonima lucida* seeds.

The different results may be due to the different tortoise species and seeds species used in the studies. However, seed dispersal is important in that seeds may escape distance and density dependent predation below parental plants and therefore have higher survival probabilities (Connel, 1970; Janzen, 1970). The study on GRT and germination experiments showed that Myanmar Star Toroise is an important disperser in the dry zone of Myanmar.So Myanmar Star Tortoises may be regarded as the important disperser in the restoration of forests in dry zone ecosystem.

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