Saurochory in the American crocodile. Are American crocodiles capable of dispersing viable seeds?

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ABSTRACT - Frugivory followed by seed dispersal is a mutualistic relationship between animals and plants. In the case of reptiles, this phenomenon has been reported in some lizards, turtles and iguanas. It has recently become apparent that frugivory is common across many species of crocodilians. However, it is unknown if crocodilians can effectively disperse seeds that are capable of germinating. To address this knowledge-gap, we tested the ability of *Leucaena lanceolata* seeds to germinate following consumption by *Crocodylus acutus*. We fed 100 seeds each to five juvenile crocodiles and then flushed each individual's stomach at specific intervals of time after feeding. We then planted the collected seeds and compared their germination rates to those of control seeds that had not been consumed by crocodiles. We found that crocodile consumption significantly lowered seed germination rates, suggesting that *C. acutus* is not an effective seed disperser.

INTRODUCTION

Crocodilian species play key roles in structuring food webs and maintaining ecological processes within tropical and sub-tropical aquatic ecosystems. For example, crocodilians can affect food web processes through predation (Bondavalli & Ulanowicz, 1999; Nifong & Silliman, 2013) and can affect population dynamics of lower trophic levels through ecosystem engineering (Craighead, 1968), as well as link multiple ecosystem types through their movement and feeding behaviours (Nifong et al., 2015; Rosenblatt & Heithaus, 2011). Traditionally, crocodilians had been thought of as strict carnivores (Grigg & Kirshner, 2015), but a recent review showed that crocodilians occasionally engage in frugivory (Platt et al., 2013a). That insight suggests that crocodilians could serve yet another role within ecosystems as seed dispersers, similar to other reptiles like lizards, turtles, and iguanas (Hnatiuk, 1978; Fialho, 1990; Moll & Jansen, 1995; Vázquez-Contreras & Ariano-Sánchez, 2016). However, the ability of crocodilians to disperse viable seeds remains largely untested.

Passage through the crocodilian digestive tract could be beneficial for seeds, as they would undergo physicochemical treatment that could enhance germination. Recently, Rosenblatt et al. (2014) tested this hypothesis with pond apple (*Annona glabra* Linnaeus) seeds consumed by American alligators (*Alligator mississippiensis* Daudin) within Everglades National Park in Florida, USA. They found that the seeds were rendered non-viable following consumption, likely because the porous nature of pond apple seed coats allowed the strong stomach acids of *A. mississippiensis* to damage the embryos. However, given that there has only been one test of the crocodilian saurochory hypothesis involving one crocodilian species and one type of seed, more research is needed to help determine if crocodilians are capable of acting as seed dispersers. Therefore, we tested the effect of seed consumption by American crocodiles (*Crocodylus acutus* Cuvier) on seed viability and germination under controlled conditions.

METHODS

For this study, we used five captive juvenile *C. acutus*, similar in body size (< 1 m) and healthy, kept individually in outdoor plastic containers (0.98 x 1.55 m, with capacity for 600 L) within the Cipactli Reptilarium facilities at Centro Universitatio de la Costa of the University of Guadalajara, Mexico. We collected mature fruits of lead trees (*Leucaena lanceolata* Watson; Family: Fabaceae) in El Salado Estuary (20°40'19.85" N, 105°14'11.35" W) located in Puerto Vallarta, Jalisco, Mexico. We used *L. lanceolata* because it has a wide distribution that overlaps with the distribution of *C. acutus* in the Pacific coastal environment of Mexico. *L. lanceolata* seeds have a semi-strong cuticle, which inhibits plant growth under natural conditions, and scarification processes increase seed germination rates (Roman-Miranda et al., 2013). *L. lanceolata* has not been reported in previous studies of the stomach contents of *C. acutus*, but some species from the family Fabaceae have been reported (see Platt et al. 2013a). In addition, the population density of *L. lanceolata* in El Salado Estuary is high (M. González-Solórzano, personal observation), therefore there is a high likelihood that *C. acutus* could consume *L. lanceolata* in this area either intentionally or accidentally.

We carried out the experiment over four weeks from October to November 2015. A total of 100 g of beef liver and fish, 40% and 60%, respectively (a detailed description in Hernández-Hurtado et al. 2012) were offered to each individual once per week. Twenty-five seeds of *L. lanceolata* were combined and amalgamated with the control diet each week, thus each individual had the opportunity to consume 100 *L. lanceolata* seeds in total. Each animal was fed ad libitum. After 24 h, the remaining food was removed and plastic containers were cleaned. The unconsumed seeds were planted in soil treated with compost and used as a control to evaluate the relative germination rate of the consumed seeds.

Stomach contents were collected from each individual using the hose-Heimlich lavage technique (Fitzgerald, 1989). In brief, this technique consists of threading a hose from the snout down to the stomach, pumping fresh water in, and then using the Heimlich maneuver to force the water and stomach contents out of the animal. The first stomach flushing was conducted at 24 hours, the second at 72 hours, and the third at 384 hours (16 days) post-ingestion. The seeds recovered from the stomachs were also planted in soil treated with compost. The germination rate of both consumed and unconsumed seeds were evaluated over 30 days. A Student's t-test was used to compare the relative amounts of consumed and unconsumed seeds among individuals. A Kruskal-Wallis ANOVA was used to compare the percentage of unrecovered and recovered seeds among individuals. A two-sample Mann-Whitney test was used to compare differences in germination rate between the unconsumed seeds and consumed seeds that were recovered by stomach flushing.

RESULTS AND DISCUSSION

Of the 500 *L. lanceolata* seeds supplied to all five individuals, 195 were consumed with a mean (± SD) of 39 ± 9.9 seeds per individual (range = 24 to 47 seeds). Of the 305 unconsumed seeds, the germination rate ranged from 7.9% to 28.3% (*mean* = 18.6 ± 8.2%). Of the 195 consumed seeds, 131 were recovered during stomach flushing, with 13 to 35 seeds recovered from each individual (*mean* = 26.2 ± 10.0). In contrast, only 8 to 16 seeds were digested or destroyed by each individual (*mean* = 12.8 ± 3.3). Thus, we found that significantly fewer seeds were digested or destroyed than those that remained intact (t = -2.85, d.f. = 8, P = 0.02). Surprisingly, there was no difference between the proportion of ingested seeds that were destroyed across stomach flushing events at different time intervals (Kruskal-Wallis test = 0.38, d.f. = 2, P = 0.82). Of the 131 seeds recovered from stomach contents, only four seeds germinated; the germination percentage ranged from 0.0% to 23.1% (*mean* = 5.2 ± 10.1%). Therefore, the two-sample Mann-Whitney test showed that a significantly lower percentage of consumed seeds germinated relative to unconsumed seeds (U = 15, P = 0.007).

In agreement with the results of Rosenblatt et al. (2014), our results provide further evidence that crocodilians may have a negative effect on seed viability. We suspect that the decrease in seed germination rates after consumption by *C. acutus* is at least in part caused by the low pH environment of *C. acutus* stomachs, which has been reported to be 3 or less (Grigg & Kirshner, 2015). We also observed that one third of the *L. lanceolata* seeds consumed by *C. acutus* were destroyed or digested, suggesting that it has a negative effect on seed viability in contrast with seed dispersers such as some birds and mammals (Castro et al., 1994; Krefting & Roe, 1949; Midgley et al., 2015), lizards (Fialho, 1990; Iverson, 1985) and both freshwater and terrestrial turtles (Hnatiuk, 1978; Moll & Jansen, 1995) which play a role as seed dispersers.

A potential issue with our study was the body size of crocodiles we used. However, although few studies have found seeds or fruits in the faeces or stomach contents of adult *C. acutus* (Alonso-Tabet et al., 2014; Alvarez del Toro, 1974; Beltrán-López, 2015; Casas-Andreu & Barrios-Quiroz, 2003; Cupul-Magaña et al., 2008; Platt et al., 2013b, 2013b, 2014; Villegas & Schmitter-Soto, 2008), seeds have been recorded in juveniles and subadults similar in size to the crocodiles in our study. This suggests that *C. acutus* may not frequently consume seeds or fruits, or that consumed seeds have a limited capacity to pass through the digestive tract. In fact, a secondary goal of this study was to estimate the excretion rate using *L. lanceolata* seeds. After feeding the crocodiles during two weeks, it was not possible to distinguish the seeds in the faeces, therefore we conducted the stomach flushing to recover the seeds and evaluate the germination rate based on the time that the seeds spent in the stomach.

Further laboratory and field tests, using different type of seeds and crocodiles of different body sizes, are needed to elucidate the functional importance of crocodilians as seed predators in tropical aquatic ecosystems.

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