

Turtle-mediated dispersal of anostracan dormant eggs: Evidence for dominance hierarchy effects

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Zoochory, or the movement of organisms by animals, can be an extremely effective means of dispersal. In freshwaters, where aquatic invertebrates can be passively spread, zoochory connects isolated and temporary waterbodies (Allen, 2007; Bohonak & Whiteman, 1999; Green et al., 2023; Vanschoenwinkel et al., 2008; Vanschoenwinkel, Gielen, Vandewaerde & Brendonck, 2008). While zoochory dynamics have been assessed for various vector taxa, the roles that the life-history stage of the vector species, and the potential hierarchical dominance of the vector, play in facilitating zoochory are yet to be assessed. Hierarchical dominance dynamics within a population results in individual characteristics, such as size and age, influencing dominance and animal behaviors. Our field and laboratory observations in a semiarid region of South Africa, in January/February 2022 provided evidence that the size of marsh helmeted terrapins (*Pelomedusa subrufa*, Order Testudines), which is related to both life-history stage and dominance, may

determine its potential to disperse dormant eggs of fairy shrimp (Subphylum Crustacea: Branchiopoda).

Various field studies have documented dominance hierarchies in freshwater testudine populations, with larger sized or older individuals being dominant over smaller or younger individuals (Harding & Bloomer, 1979; Keevil et al., 2017; Masin et al., 2020). We opportunistically tested for the potential of size-related dominance hierarchy in *P. subrufa* to contribute to the dispersal of large branchiopod dormant eggs in a southern African temporary wetland complex. On 12 January 2022, while sampling a wetland site as part of a larger investigation of temporary pan ecology, it was evident that high numbers of testudines were present. Dozens of *P. subrufa* were seen breaching the water surface with their heads at any one time. Sampling of the pan also revealed high densities of fairy shrimp. As such, we collected terrapins (Figure 1A–C) of two discrete size classes (small and large) and obtained fecal

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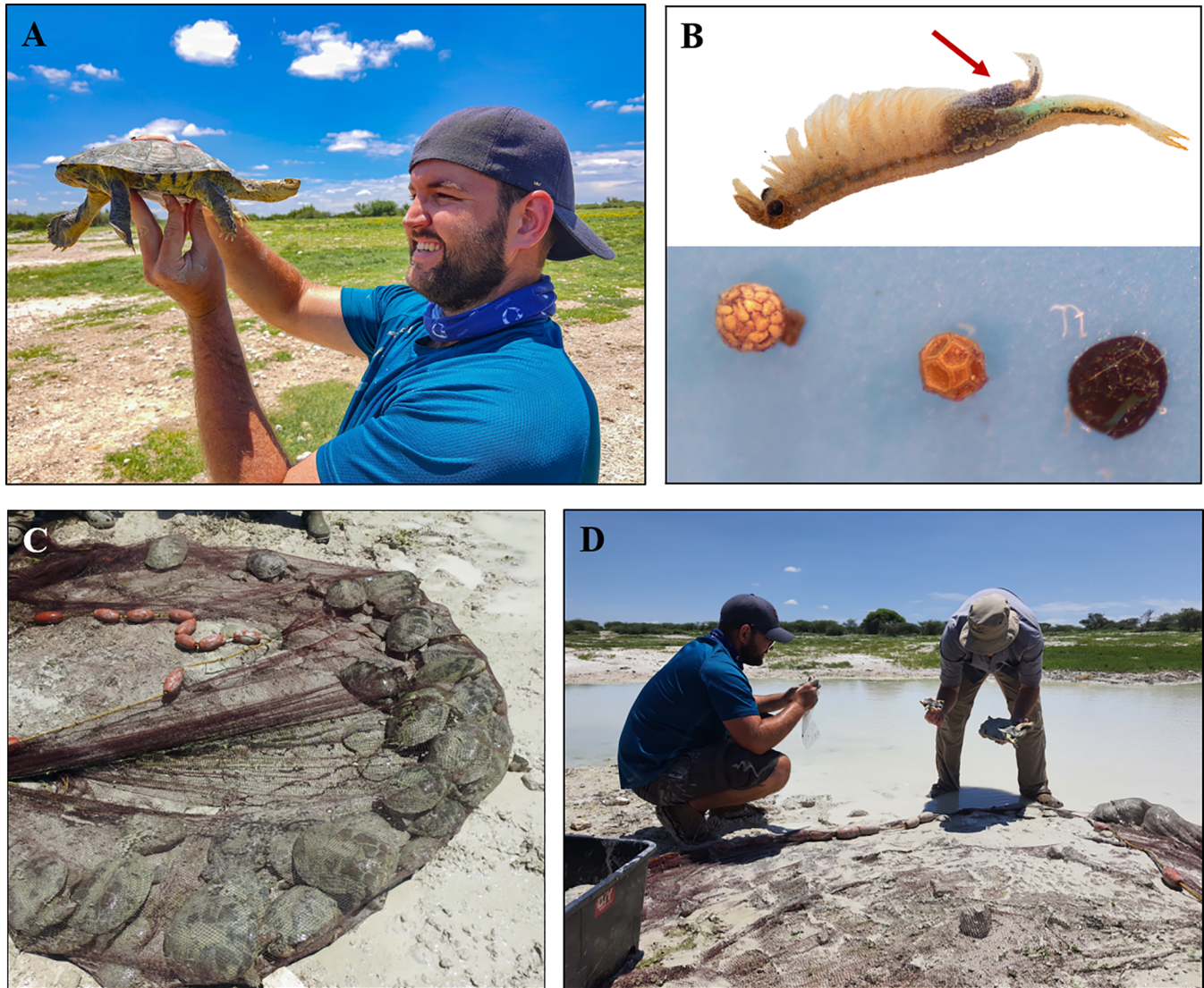


FIGURE 1 Here, author Chad Keates is inspecting (A) a marsh helmeted terrapin *Pelomedusa subrufa* sampled from the study pan. During the rainy season, *P. subrufa* colonize temporary pan ecosystems and feed on fairy shrimp (B), including females containing dormant eggs in their brood pouches (maroon arrow). Terrapins move between pans, potentially spreading dormant eggs and maintaining fairy shrimp dispersal between isolated populations. At the study pan, a single seine net haul produced 61 *P. subrufa* individuals (C). Fecal samples were successfully collected from individuals (D) for processing in the laboratory, whereby *Streptocephalus* spp. dormant eggs (B-upper) were identified and assessed for viability using a hatching protocol. Fairy shrimp dormant eggs from *Streptocephalus* cf. *ovamboensis* (B – bottom left) and *Streptocephalus* cf. *indistinctus* (B – bottom center) were found, along with an unidentified “other” egg-like item (B – bottom right). Photographs (A), (C) and (D) by Tatenda Dalu. Photographs in (B) are of fairy shrimp by Chad Keates and eggs by Ryan Wasserman.

samples to assess for the presence of large branchiopod dormant eggs (Figure 1D), their viability through hatching experiments, and size-related differences in zoochorous potential.

In small endorheic temporary wetlands, biological communities are typically comprised of both fully and semi-aquatic organisms (Bird et al., 2019; Dalu & Wasserman, 2022). In arid regions, the fully aquatic component is often dominated by crustaceans, most of which produce dormant eggs for reproductive purposes (Brendonck et al., 2022).

These include large branchiopods, which typically do not employ direct development strategies, relying entirely on dormant egg production to facilitate population persistence in temporary aquatic ecosystems. These dormant robust eggs are capable of withstanding desiccation for prolonged periods, facilitating their persistence in harsh aquatic ecosystems while favoring dispersal potential (Vanschoenwinkel et al., 2011). Given that freshwater habitats are typically embedded within a terrestrial matrix, fluxes between terrestrial and aquatic

environments, and among discrete aquatic habitats by faunal groups are catholic (Cuthbert et al., 2022; Knight et al., 2005). This is particularly relevant for mosaics of smaller water bodies, where metacommunity dynamics are characterized by a relatively large number of separate aquatic populations (Cuthbert et al., 2022; Gálvez et al., 2022).

Of the semiaquatic organisms that inhabit temporary wetlands, testudines have the potential to move dormant eggs between waterbodies. They have the propensity to disperse overland after consuming large branchiopods with dormant eggs in their brood pouches (Boycott & Bourquin, 2008). Following our observation of co-occurring large densities of fairy shrimp with marsh helmeted terrapins (see study site details in Appendix S1), we hauled a seine net (10 m long, 1.5 m wide, 1 cm mesh size) through the deepest point in the pan, covering an area of ~20% of the pan size. In total, 61 terrapins were sampled from the single seine net haul, all identified as *P. subrufa*. Two size classes were evident, with 36 individuals categorized as small (65–115 mm carapace length) and 25 individuals (190–220 mm carapace length) as large. No *P. subrufa* individuals between 115 and 190 mm were sampled, suggesting that the two size classes represented discrete age groups. It was observed that while taking size measurements upon handling, some *P. subrufa* would defecate. The feces were carefully and opportunistically collected by placing a clean, inverted Ziplock bag under the cloaca to catch the defecant as it was extruded, being careful not to allow contact with any sediment or the animal's body. Each individual was held for no longer than 60 s before being gently released back into the wetland. Fecal matter was attained from 14 individuals in total: Eight individuals of the smaller size class and six individuals of the larger size class. Ziplock bags with fecal samples were then placed in a large polystyrene cooling container and allowed to air dry for 7 days prior to laboratory processing.

The dried fecal samples were gently crushed in the Ziplock bags, with the contents then gently transferred into a weighing boat and weighed to the nearest 0.1 mg, using an Ohaus Adventurer Balance, model AR2140. All statistical analyses were done using R version 4.1.1 with the *base* package Wilcoxon rank sum tests (R Core Team, 2021). The Brown–Forsythe test in R using the *onewaytests* package (Dag et al., 2018) was used to test for significant differences in the homogeneity of variance. For both dry fecal mass and potential dormant egg density, there was no significant difference in variances between size classes (both $p > 0.05$). Using the Wilcoxon rank sum test, no significant differences ($W = 37$, $p = 0.11$) in dry fecal sample mass between the two size classes was detected (Figure 2A). Samples were then carefully transferred to a zooplankton counting tray for

processing using a dissecting microscope (Olympus SZ61) at $\times 20$ magnification to assess for potential dormant eggs. Items with external morphological features that looked like dormant eggs were counted per morphotype and expressed as density of items per unit dry fecal mass. Fecal samples from all eight small-sized and two large-sized individuals contained dormant eggs from branchiopod crustaceans (Figure 2B). All large-sized individuals also contained items with external ornamental patterns similar to dormant eggs, but these could not be discerned from seeds or parasitic cysts that can show similar features (Figure 2B).

The densities of items (potential dormant eggs) in feces varied significantly between *P. subrufa* size classes ($W = 1$, $p = 0.001$), with the small size-class feces holding more dormant eggs (Figure 2B). The two dormant egg morphotypes were both identified as anostracans of the genus *Streptocephalus* (Figure 1D). These were designated as *Streptocephalus* cf. *indistinctus* and *Streptocephalus* cf. *ovamboensis* (Brendonck & Coomans, 1994; Hamer & Brendonck, 1993). The unidentified morphotypes had no identifiable features and were thus classified as “other.” Within the small size-class samples, 84.6% of items of interest were identified as dormant eggs of *Streptocephalus* cf. *indistinctus*, 12.8% as dormant eggs of *Streptocephalus* cf. *ovamboensis* and 2.6% as the “other” morphotype. Within the large size-class samples, 95.1% of items of interest were the “other” morphotype, with 4.9% verified as dormant eggs of *Streptocephalus* cf. *indistinctus*, and no *Streptocephalus* cf. *ovamboensis* dormant eggs sampled from this size class (Figure 1C).

A subsample of dormant egg morphotypes was isolated from the fecal matter of each size class of *P. subrufa* to assess viability after passing through the gut of the potential zoochorous agent. One liter of distilled water was used to make a hatching water solution with a conductivity of $\pm 340 \mu\text{S cm}$ (see physico-chemical characteristics in Appendix S2). Following the procedures outlined in Tladi et al. (2020), a dormant egg was isolated and gently transferred from the zooplankton counting tray, using a fine needle tip, to an individual glass vial (22 mm \varnothing), filled and maintained with 20 mL of the water solution. Given differences in dormant egg morphotype and “other” item numbers attained per *P. subrufa* size class, different numbers of these items were used for hatching assessment. For the small size class, 28 dormant eggs of the morphotype “*Streptocephalus* cf. *indistinctus*” and 13 of the dormant eggs of the morphotype “*Streptocephalus* cf. *ovamboensis*” were used for hatching. Only four of the “other” items were used for the small size-class hatching. The hatching success of the dormant eggs from the small-sized terrapin feces was 64.3% and 38.5% for *S. cf. indistinctus* and *S. cf. ovamboensis*, respectively. These

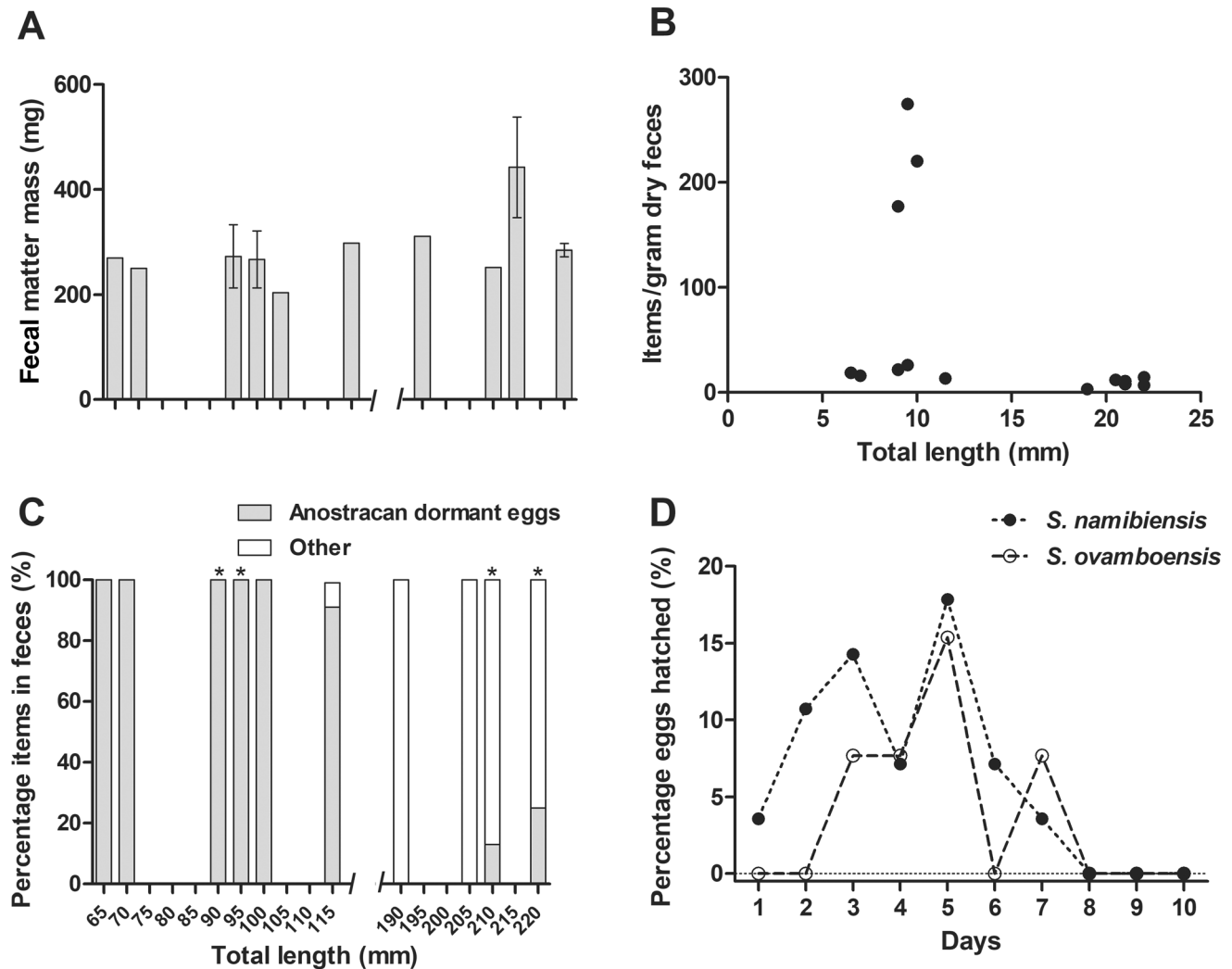


FIGURE 2 Compound figure outlining (A) fecal mass of individual *Pelomedusa subrufa* across different sizes (mean \pm SD presented where two individuals of the same size occur); (B) densities of all potential dormant eggs within fecal matter across differently sized *P. subrufa*; (C) percentage of anostracan dormant eggs versus “other” unidentified items across different sized *P. subrufa* (star where mean of two individuals of the same size presented); and (D) hatching success of fairy shrimp species dormant eggs over time, from small size class (65–115 mm) *P. subrufa* fecal samples. (A) and (C) share the same x axis details, and the x axes are split on (A) and (C).

fairy shrimp dormant eggs all hatched within 7 days, with the majority hatching between days 3 and 5 for both species (Figure 2D). None of the “other” unidentified items hatched from the small-size class. For the large size class, two dormant eggs of the morphotype “*Streptocephalus* cf. *indistinctus*” and 10 items of the morphotype “other” were used for hatching. None of these eggs hatched successfully. Because no “*Streptocephalus* cf. *ovamboensis*” dormant eggs were present in fecal samples from large-sized *P. subrufa*, hatching success from this morphotype could not be assessed for this size class.

The densities and hatching success of dormant eggs from the *P. subrufa* fecal samples highlighted the potential for freshwater testudines to facilitate population connectivity between discrete wetland types, but also that

size of terrapin is an important consideration. It is likely that anostracan dormant eggs were consumed along with adult females, as sexually mature *Streptocephalus* spp. carry large numbers of viable eggs within their brood pouches (Figure 1B). Indeed, temporary pans are good foraging patches for predators, given their high levels of secondary production, which are often driven primarily by large branchiopods (Brendonck et al., 2022). *Pelomedusa subrufa* exhibit predatory behavior and consume invertebrates when available, and invertebrate remains were also detected in the fecal samples when screening for dormant eggs. Similarly, during rock-pool surveys in Central District Botswana in the rainy season of 2019, author Ryan J. Wasserman encountered an individual *P. subrufa* in a very small rock pool alongside large

numbers of the anostracan *Branchiopodopsis tridens*. The following day, the same rock pool was visited, and the terrapin was no longer present, and very few *B. tridens* remained (Wasserman, personal observations). These observations, along with the results of the present study, suggest that temporary wetlands may serve as attractive foraging habitats for *Pelomedusa subrufa*. However, the differences in dormant egg densities between large-sized and small-sized *P. subrufa* suggest that size-structured effects on zoochorous potential for the species exist. Smaller sized individuals contained significantly higher densities of both species of fairy shrimp dormant eggs and lower numbers of the “other” dormant egg type. These size-structured differences may reflect dietary preferences between small and large terrapins, whereby individuals are mainly carnivorous during juvenile stages before switching toward omnivory in later life (Ernst & Lovich, 2009). Hatching failure of the “other” items suggests that either they were not dormant eggs, or that gut conditions in large-sized terrapin may be less hospitable and reduce egg viability.

The other component, not assessed here but equally important regarding zoochory, is the movement of the vector species. Given the impermanent nature of temporary wetlands, there is inherent pressure for semiaquatic generalist species to move across landscapes in search of water as pans dry. During periods of rain, *P. subrufa* travels for large distances across dry land, as much as 5 km or more in just 3 days (Stuart & Meakin, 1983), occupying and moving between even very small temporary pools (Boycott & Bourquin, 2008). Testudine dominance hierarchy effects may also come into play with regard to movement between freshwater bodies. Dominance hierarchy theory postulates that, for population life-history groups that exploit similar niches, hierarchical dominance will arise (Werner & Gilliam, 1984). In higher organisms, emergent hierarchies are typically size-related, with smaller individuals subordinate and likely to be marginalized (Werner & Gilliam, 1984). Various field studies have highlighted that dominance hierarchies indeed establish in freshwater testudine populations, with larger or older individuals being dominant over smaller or younger individuals (Harding & Bloomer, 1979; Keevil et al., 2017). As this relates to habitat use in small temporary aquatic systems, this process probably has implications for pressure to move from an aquatic habitat, with increased pressure for smaller individuals to move in search of alternative aquatic habitats. For example, juveniles of Sonoran mud turtles *Kinisternon sonoriense* exhibit increased movement between disconnected freshwater habitats in Arizona, USA (Hall & Steidl, 2007). Therefore, a dispersive synergy may form, whereby smaller subordinate individuals are more likely to consume fairy shrimp due to dietary

preferences while also showing higher mobility than larger dominant terrapins.

This study highlights that freshwater testudines are good candidates for the endozoochory of large branchiopods, given that they ingest dormant eggs which make their way through the gut while remaining viable. *Pelomedusa subrufa* may be particularly important in temporary wetland zoochory given their propensity to move large distances in search of remote temporary waterbodies, unlike other closely related terrapins that prefer permanent waterbodies (Boycott & Bourquin, 2008). Future studies should investigate the direct association of terrapins with predation, gut retention times, and the potential for external adherence of dormant eggs to terrapins in dry periods. These studies should involve designs that allow for more natural gut evacuation dynamics, as it could be argued that defecation in the present study was premature due to the handling of the individuals, with implications for gut retention times and dormant egg viability. Using tracking systems in landscapes with mosaics of temporary wetlands, dispersal frequency, timing and distance dynamics in relation to different *P. subrufa* size and dominance classes, also need to be investigated. These data could be modeled to determine propagule number and distance export dynamics by turtles. Similarly, vector dormant egg loads, vector mobility, and dormant egg gut passage time could be used to model differences quantitatively in dispersal capacity among other zoochorous taxa such as large mammals, wading birds, amphibians and reptiles.

AUTHOR CONTRIBUTIONS

Ryan J. Wasserman identified the project and sampling design, contributed to funding, executed the field sampling, conducted the laboratory analyses and produced the first draft of the document. Ross N. Cuthbert identified the project and sampling design, conducted statistical analyses and contributed to the writing. Tatenda Dalu and Farai Dondofema identified the project and sampling design, contributed to funding, executed the field sampling and contributed to the writing. Chad Keates executed the field sampling and contributed to the writing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Wasserman, 2023) are available in Dryad at <https://doi.org/10.5061/dryad.1g1jwsv22>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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