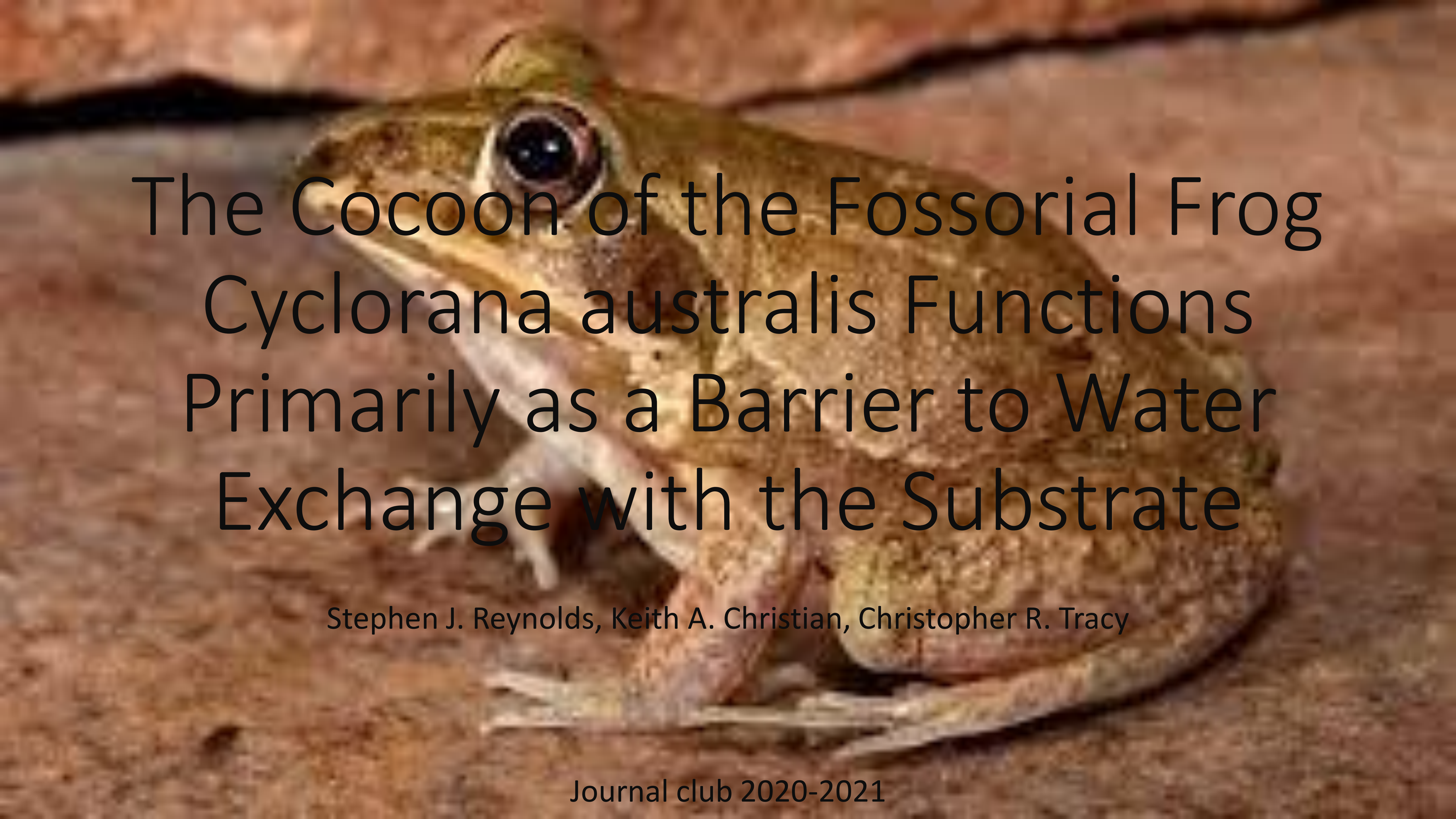

The Cocoon of the Fossorial Frog Cyclorana australis Functions Primarily as a Barrier to Water Exchange with the Substrate

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INTRODUZIONE:

- Cyclorana australis, or Litoria australis, or giant frog is a large (60–100 g) member of the genus from monsoonal northern Australia, is a fossorial frog and forms a cocoon during the annual dry season, from May to October;
- Cocoon is composed of shed layers of squamous epithelia, is a sort of dried skin, becomes thicker as new layers are formed and gradually hardens to encase the entire body except the external nares;
- In this study we want to explain that the frog cocoon has a recognized role in reducing evaporative water loss (EWL) from the skin surface and acts as a barrier to water exchange with the soil, breaking the continuity between the skin and the substrate.

INTRODUZIONE:

From previous studies:

- microclimatic conditions and physical characteristics of the burrows;
- before the cocoon has formed the ventral surface of C. australis is in contact with the soil substrate, and the exchange of liquid water with the soil is dependent on the water potential (WP) of the frog and on the matric potential;
- with the presence of the cocoon the ventral surface of the skin is not in contact with the soil;
- the rate of water flux depend on: the WP difference, the permeability of the (ventral) skin and the surface area in contact with the substrate, mediated in part by the action of neurohypophysial hormones;

ESPERIMENTO:

- In laboratory we compared the influence of the cocoon of C. australis on liquid water exchange with the substrate, between cocooned frogs and frogs without cocoons.
- We used a semisolid agar osmoticum to tightly control WP and emulate the soil surface.
- We measured the osmolality of the body fluids in cocooned, decocooned frogs and hydrated frogs.
- We also investigated the rate of EWL over time in cocooning frogs to determine the time required for development of substantial resistance to water flux.

MATERIALI E METODI:
ANIMALI E BOZZOLI

- Animals were obtained during the wet season from sites in the vicinity of Darwin (12°24’S, 130°50’E), Northern Territory, Australia
- Before forming a cocoon, the frogs were fully hydrated
- During cocoon formation, the frogs were maintained in the laboratory in darkened, ventilated containers at 24°+/- 1°C and a relative humidity of 45%–60%
- All frogs formed cocoons for 8 or more weeks(10) to ensure that the cocoon had sufficient thickness→high resistance to EWL

MATERIALI E METODI:
SCAMBIO IDRICO

- A 1% solution of agar (1 g agar per 100 mL water) was prepared with varying concentrations of sucrose or polyethylene glycol (PEG) 1000: PEG 1000 was chosen because soluble in water but with high molecular weight, so not permeable to frog skin, instead sucrose is highly soluble and with a lower molecular weight (342 Da) but has more predictable osmotic effects.
- The total WP of the agar+solute substrate was calculated as the combined matric (due to agar; estimated as -210 kPa) and osmotic (due to dissolved solute) WPs.
- The frog was placed in a container with an agar+PEG or agar+sucrose substrate.
- The mass of the frog placed on the substrate for 10 s was taken as the initial mass, then the trial was begun. Mass was measured at 15-min intervals over a 90-min period.
- Water exchange was the total mass gain or loss over the 90 min, converted to hourly rates.

MATERIALI E METODI:
SCAMBIO IDRICO

- The first trial was of cocooned frogs, obtained from the darkened containers in which they had been forming cocoons
- At the end of the 90-min trial, the frog was removed from the substrate, the cocoon was detached by making an incision on the dorsal surface and peeling the dried skin away from the frog; cocoons were weighed, and oven dried at 60°C for 5 days
- The second trial was of frogs with cocoons removed, they were then placed on a newly prepared substrate at the same WP and following the same weighing procedures
- Then frogs were rehydrated and after 5 days with access to water, the bladders were drained to give a measure of standard mass
- The third trial was of hydrated frogs: to ensure full hydration, frogs were placed in shallow water overnight; trials were carried out the following morning, after emptying the bladder

MATERIALI E METODI:
OSMOLALITÀ DEI FLUIDI CORPOREI

- Body fluids in the second trial: urine was obtained by inserting a flexible plastic cannula into the cloaca, plasma samples were obtained from the femoral lymph sac using a fine-gauge needle. In C. australis blood plasma and lymph are equivalent
- Body fluids in hydrated animals: urine was obtained as the bladder was being emptied before trials, and plasma was obtained at the end of the trial
- Osmolality of body fluids was measured with a micro-osmometer: frog WP is related to plasma osmolality by the equation: WPfrog=-CRT

RISULTATI:
cocooned animals

- Cocoons: intact, well formed, dry to the touch and parchment like;
- Frogs inside were immobile;
- At the end of the experiment: cocoon was moist to the touch over the ventral surface and quite supple; in some cases it began to break down along the midline of the belly, whereas the dorsal cocoon remained dry and inflexible;
- Cocooned frogs showed a relatively low and constant increase in mass (range 5.5–14.6 mg/cm-h), irrespective of the WP;
- None of the cocooned frogs lost water to the substrate even at very low WPs→cocoon formation reduces water loss

RISULTATI:
noncocooned animals

- Noncocooned animals on substrates with high WP press their pelvic patch area against the substrate;
- Water uptake was rapid on pure agar (137 mg/cm^2,h) but decreased on substrates (agar+sucrose and agar+PEG) with low WP;
- Only 2 decocooned frogs, at very low WPs, lost water; but most decocooned frogs that had relatively high plasma osmolality, absorbed water;

RISULTATI: hydrated animals

- Hydrated frogs on substrates of very low WP made scraping movements on the surface of the agar with their hind limbs and, in some cases, lifted their fingers and toes above the substrate;
- Uptake was possible on substrates with WP greater than approximately -600 kPa (equivalent to 250 mOsm/Kg), but for more negative values all frogs lost water;

RISULTATI:

- Plasma and urine osmolality were not significantly different in the sucrose and the PEG groups for decocooned and hydrated animals
- Osmolality of the plasma and urine of cocooned frogs was significantly higher than in hydrated frogs;

Table 1: Osmolality of body fluids of cocooned and rehydrated *Cyclorana australis* used in water exchange trials

Group	Plasma Osmolality ($mOsm\ kg^{-1}$)	n	Equivalent Mean Frog WP (kPa)[a]	Urine Osmolality ($mOsm\ kg^{-1}$)	n
Cocooned	427.3 ± 73.6	9	−1,040	439.2 ± 95.6[b]	10
Rehydrated	234.4 ± 12.2	10	−570	45.0 ± 31.6	10

Note. Osmolality values are means ± SD. n, sample size; WP, water potential.

[a] Calculated from mean plasma osmolality at 20°C.

[b] The reported mean osmolality for urine is higher than that for plasma because there was insufficient plasma for measurement in one of the cocooned frogs, and this individual had an elevated urine osmolality (with this individual excluded, $n = 9$ and mean urine osmolality = 421.9 $mOsm\ kg^{-1}$). The urine and plasma means are not significantly different, and in all cases the urine of an individual frog was hyposmotic to or isosmotic with plasma.

RISULTATI:

- In cocooned frogs, during the cocoon formation, there was a relatively rapid initial increase in EWL, followed by a gradual reduction over the follow weeks; after, water loss was relatively constant at ~0.2 mg/g,h for cocooned frog, instead for hydrated frogs water loss was of 1.2-1.7 mg/g,h;

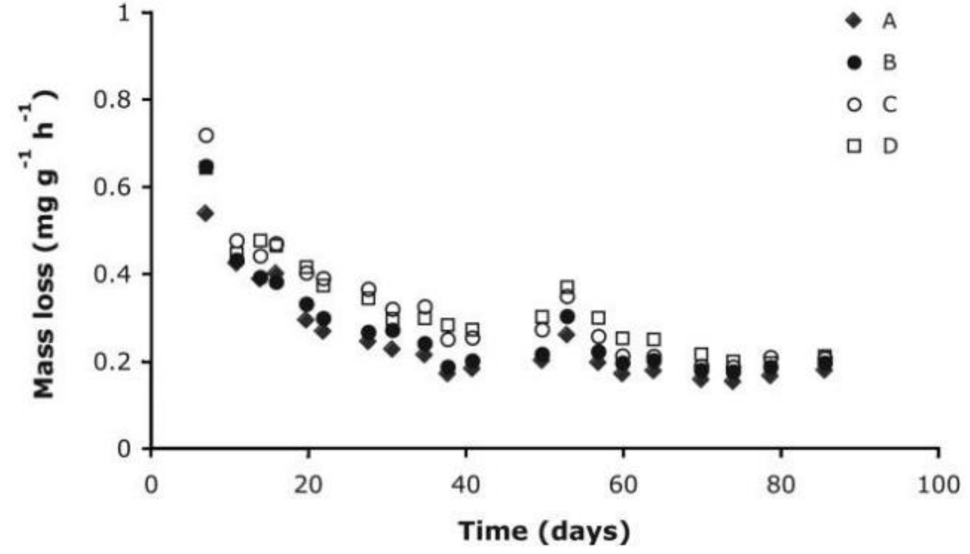

Figure 2. Mass loss over time during cocoon formation in *Cyclorana australis* ($n = 4$). Individual animals (A–D) without access to water were maintained in the laboratory and weighed periodically. Note that the first measurement was taken after 7 d, when cocoon formation had already begun. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology*.

DISCUSSIONE:

- The difference in mass between a freshly removed and an ovendried cocoon was between 59% and 88% of the increase in mass: this suggests that moisture was absorbed by the multiple epidermal layers of the cocoon, infiltrating the fibrous matrix and causing expansion of the layers;
- The physical separation of the frog skin from the substrate means that, regardless of the direction and magnitude of the WP difference, there is no physiological exchange;
- The relationship between WP and relative humidity (RH) is logarithmic, so that at WPs of -570 kPa, corresponding to the body fluids of a hydrated frog, equivalent RH is greater than 99%;

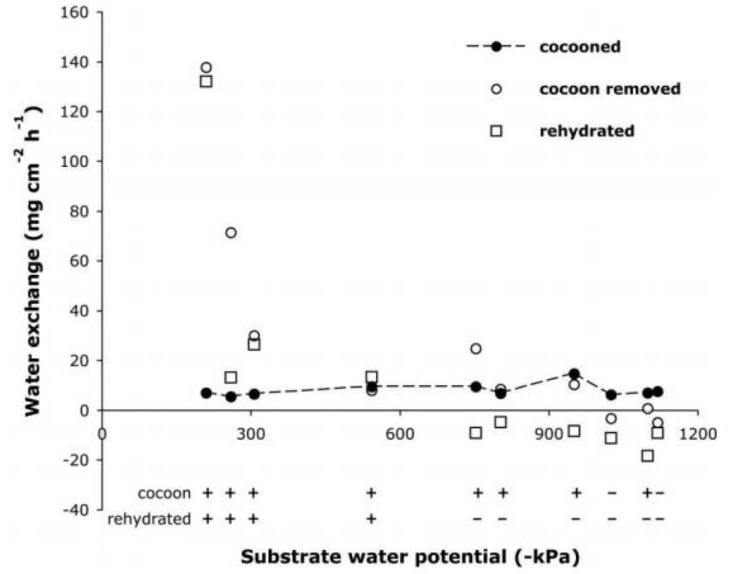

Figure 1. Water exchange of *Cyclorana australis* (n = 10) on semisolid substrates of agar + solute at various water potentials. Experimental procedures were carried out in the laboratory at 24° ± 1°C. The frog cocoon (*filled circles*) absorbed consistently low quantities of water, regardless of substrate water potential. Water exchange in frogs without cocoons was dependent on the water potential difference between frog and substrate. For frogs with the cocoon removed, a plus sign indicates that the water potential difference ($\psi_f - \psi_s$, where ψ_f is calculated from plasma osmolality and ψ_s is based on agar + solute) favored movement of liquid water from the substrate to the frog, whereas a minus sign indicates that the gradient was in the opposite direction.

- Water uptake in hydrated frogs occurred on substrates with WPs greater than -300kPa (=120 mOsm/Kg), but water was lost to substrates with WPs lower than -600kPa (=250 mOsm/Kg);
- At relatively high WPs of >-100 kPa frogs are able to absorb water from the soil;
- At lower WPs, when the bulk water has drained from the soil, frogs lose water from the skin surface;

DISCUSSIONE:

- The intact cocoon forms an effective barrier on dry substrates, whereas on wet substrates the cocoon would eventually soften and split open.
- The substrate used in the experiments most closely resembles a clay soil, in which water is retained even at low WP; the agar substrate maintains hydraulic conductivity because water movement is unrestricted within the interstices of the gel matrix;
- Clay soils retain moisture at low WPs so that cocoon formation may coincide with development of a WP gradient;
- This substrate is also useful to understand behavior associated with water uptake, such as the water absorption response→ some frogs adopted a distinctive posture where the digits were lifted off the substrate maybe to reduce the loss of water

DISCUSSIONE:

- In cocooned frogs, there may be a space between the skin and the cocoon, which would prevent mass transfer of liquid water from skin to cocoon;
- The accumulated cocoon layers retard the passage of water molecules evaporating from the (saturated) skin surface to the air inside the burrow chamber: with an increase in epithelial layers, resistance to EWL increases; cocoon acts itself as a barrier for liquid water and also for evaporative water loss(EWL);
- The ability to form a cocoon may eliminate the necessity of constructing deep burrows and allow fossorial frogs to use drier parts of the landscape;
- We can assume, from this experiment that the cocoon is an adaptation to a subterranean existence and that it has evolved largely to reduce rates of water loss (liquid and vapor) to dry soils.


GRAZIE PER L’ATTENZIONE!