# RESSALVA

Atendendo solicitação da
autora, o texto completo desta tese
será disponibilizado somente a partir
de 17/08/2020.

UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(ZOOLOGIA)

# O ovo flexível: influência da água durante a incubação de *Iguana iguana*

Laura Borelli Thomaz Carreira

Agosto - 2018

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)**

# O ovo flexível: influência da água durante a incubação de *Iguana iguana*

**Laura Borelli Thomaz Carreira**

**Orientador: Augusto Shinya Abe**

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas (Zoologia).

**Agosto - 2018**

C314o

Carreira, Laura Borelli Thomaz
O ovo flexível: influência da água durante a incubação de Iguana iguana / Laura Borelli Thomaz Carreira. -- Rio Claro, 2018
117 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientador: Augusto Shinya Abe

1. Fisiologia Comparada. 2. Embriologia de Réptil. 3. Potencial Hídrico. 4. Diagnóstico por Imagem. 5. Morfologia Embrionária. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Rio Claro. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

**Câmpus de Rio Claro**

## CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: O ovo flexivel: influência da água durante a incubação de Iguana iguana

**AUTORA: LAURA BORELLI THOMAZ CARREIRA**
**ORIENTADOR: AUGUSTO SHINYA ABE**

Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), pela Comissão Examinadora:

Prof. Dr. AUGUSTO SHINYA ABE
Departamento de Zoologia / Instituto de Biociências de Rio Claro - SP

Prof. Dr. WILFRIED KLEIN
Departamento de Biologia / USP - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto / SP

Prof. Dr. LUIZ HENRIQUE FLORINDO
Departamento de Zoologia e Botânica / UNESP - Câmpus de São José do Rio Preto

Prof. Dr. CLEO ALCANTARA COSTA LEITE
Departamento de Ciências Biológicas / UNIVERSIDADE FEDERAL DE SÃO PAULO - UNIFESP

Prof. Dr. JOSE EDUARDO DE CARVALHO
Ecologia e Biologia Evolutiva / Universidade Federal de São Paulo - UNIFESP

Rio Claro, 17 de agosto de 2018

UNIVERSIDADE ESTADUAL PAULISTA

unesp

**Câmpus de Rio Claro**

**ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE LAURA BORELLI THOMAZ CARREIRA, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), DO INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE RIO CLARO.**

Aos 17 dias do mês de agosto do ano de 2018, às 14:00 horas, no(a) Sala da Congregação - Prédio da Administração/IBRC, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Prof. Dr. AUGUSTO SHINYA ABE - Orientador(a) do(a) Departamento de Zoologia / Instituto de Biociências de Rio Claro - SP, Prof. Dr. WILFRIED KLEIN do(a) Departamento de Biologia / USP - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto / SP, Prof. Dr. LUIZ HENRIQUE FLORINDO do(a) Departamento de Zoologia e Botânica / UNESP - Câmpus de São José do Rio Preto, Prof. Dr. CLEO ALCANTARA COSTA LEITE do(a) Departamento de Ciências Biológicas / UNIVERSIDADE FEDERAL DE SÃO PAULO - UNIFESP, Prof. Dr. JOSE EDUARDO DE CARVALHO do(a) Ecologia e Biologia Evolutiva / Universidade Federal de São Paulo - UNIFESP, sob a presidência do primeiro, a fim de proceder a arguição pública da TESE DE DOUTORADO de LAURA BORELLI THOMAZ CARREIRA, intitulada **O ovo flexivel: influência da água durante a incubação de Iguana iguana**. Após a exposição, a discente foi arguida oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof. Dr. AUGUSTO SHINYA ABE

Prof. Dr. WILFRIED KLEIN

Prof. Dr. LUIZ HENRIQUE FLORINDO

Prof. Dr. CLEO ALCANTARA COSTA LEITE

Prof. Dr. JOSE EDUARDO DE CARVALHO

**Acknowledgements**

To the Post-Graduate Program in Biological Sciences (Zoology) of UNESP-Rio Claro, for the opportunity;

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, for the financial assistance;

To my supervisor, Prof. Dr. Augusto Shinya Abe, for all the teachings transmitted;

To the Post-Graduation Sections of UNESP-Rio Claro, ESALq-Piracicaba and USP-São Carlos, for all the commitment and collaboration;

To the professors of Esalq: Prof. Dr. Marcos Vinícius Folegatti; Prof. Dr. Francisco André Ossamu Tanaka and Prof. Dr. Alvaro Pires da Silva (*in memoriam*); that somehow contributed to the accomplishment of this work;

To Prof. Dr. Alberto Tannús and the doctorate Agide Marassi, for all the support, collaboration and teachings at the Physics Institute of USP-São Carlos;

To my family. Especially my mother, Márcia Mondin Thomaz, for all the support, understanding, affection and love throughout this period. Understanding my absences in the Christmas and New Year periods, because they always coincided with the breeding and laying eggs seasons and experiments in the Laboratory. In addition to the countless advice given in difficult times and shared joy in happy times.

To the dear and loved friends, who have always been on my side giving support, affection, understanding and strength. In particular:

-Marina Rincon Sartori, who had an essential role in my training during the course; transmitting teachings, giving advice and guidance, always with great affection, friendship and understanding. Sharing experiences, moments of relaxation, joy and also difficulties. Thank you for teaching me about compliance, not only with regard to the eggs, but mainly for showing me this on a day to day basis and helping me become a better person.

-Guilherme Gomes, who was present in various moments of the Doctorate (from friend to evaluator!). Always supporting me, teaching, helping; independently of the geographic distancing. Thank you for all of this, and perhaps mainly for helping me to see beyond the pattern and to look for new things, even in totally different areas, but which can be interconnected if you look closely.

-Renato Filogonio, for being my first friend in the Jacarezário. Always transmitting calm and lightness, discussing with me research results, new ideas, old ideas, helping in times of despair (yes, I already understood that having a cup of coffee and breathing deeply improves 50% of our capacity to solve the problems.). Thank you also for teaching me how to enjoy every moment of my work, being it good or bad. That made a lot of difference! Besides, of course, to have improved my musical taste and playlists. In addition to the great movies and books indications.

-Laura Pozzo, my dear twin of name and heart. For all the affection, support, shoulder, friendship and companionship, during those years in Rio Claro.

-Nathália Oliveira Goes Silva, who for more than 20 years has had my infinite friendship. Always supporting me, giving advices, sharing moments and experiences, making my days happier.

- Tatiane Tuha and Yumi Maeda, for the friendship, affection, support and companionship, during those years in Rio Claro.

To the dear Sergio Zumpano Arnosti, for all the affection, love, patience, understanding and mutual teachings exchanged. To the learning and personal growth provided by the acquaintanceship and dialogue.

To my psychologist, Igor Salomão Monteiro, for all the support and commitment to help me overcome traumas, onset of depression and anxiety disorders throughout the PhD course, and always caring a lot about my mental health. Undoubtedly had a fundamental role in this work, because without his guidance I would not have come so far.

To my psychiatric doctor, Fanny Kaori Usami, for all the commitment to make me feel better during this walk, always taking care of my mental health and providing conditions so that I could fulfill this work.

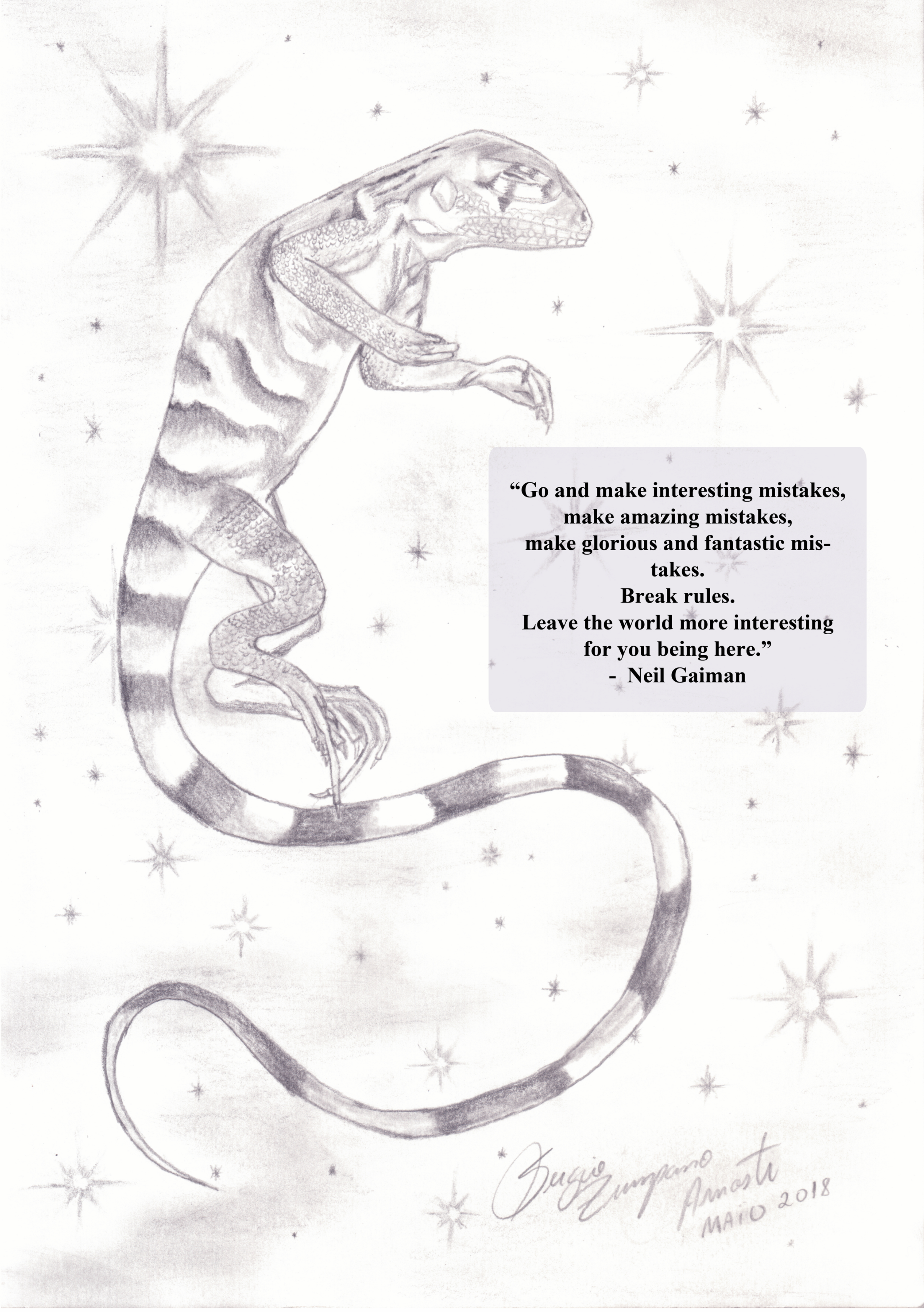
To the technicians of Jacarezário Ayrton Nascimento and Fernando Roza, for all the effort, work, cooperativism and friendship that made possible this study.

To the technician of the Department of Zoology Luiz Fernando Bonatti, for all the aid, cooperativism and friendship.

To the dear Edna, who with great affection and good humor makes our days lighter in the laboratory.

To the technician of microscopy Antônio Teruyoshi Yabuki, for all the commitment, teachings, cooperativism and friendship in the processing of materials and acquisition of images of scanning electron microscopy.

To all my students of the Biological Sciences course, who taught me a lot about teaching classes, diversity of ideas and personal growth.




**“Go and make interesting mistakes,**
**make amazing mistakes,**
**make glorious and fantastic mis-**
**takes.**
**Break rules.**
**Leave the world more interesting**
**for you being here.”**
**- Neil Gaiman**

**“This is how you do it:**

**You sit down at the keyboard and**

**you put one word after another until its done.**

**It's that easy, and that hard.”**

- **Neil Gaiman**

## Resumo

Os ovos de cascas flexíveis são ausentes de reserva hídrica provida pela mãe, e necessitam absorver água, necessária para o seu metabolismo, através do meio de incubação que, em condições naturais, é diretamente influenciado pelo regime de chuvas. Desta forma, com o objetivo central de entender a fisiologia embrionária de répteis que possuem ovos de casca flexível, foram utilizados ovos de iguana verde (*Iguana iguana*) incubados sob diferentes potenciais hídricos variando de extremamente seco para extremamente úmido, onde foram avaliados parâmetros que refletem a fisiologia do balanço hídrico destes embriões: osmolalidade, massa seca, conteúdo de água e pressão interna dos ovos. Além disso diagnósticos por imagens de microscopia eletrônica de varredura e ressonância magnética também foram aplicados com o intuito de verificar a morfologia da casca destes ovos e a avaliação não invasiva do desenvolvimento embrionário sob diferentes condições hídricas, respectivamente. Dentre os principais resultados encontram-se um aumento bastante significativo da concentração osmótica de fluido alantóico em ambiente extremamente seco resultando em 385 mOsmol/kg. As taxas de massas mostram que os embriões exibem um aumento de 158% em tratamentos extremamente secos quando comparados com tratamentos úmidos. Em condições secas os ovos apresentaram deformação da casca e o embrião representou a maior parte do ovo inteiro, com frações muito pequenas de vitelo e alantoide. A espessura da casca varia conforme a condição hídrica, podendo apresentar uma redução superior a 50%. Além disso, existe um desgaste da casca do ovo ao final da incubação resultante principalmente da ação hídrica, além da degradação por microrganismos, e não da mobilização de cálcio para o embrião conforme ocorre em ovos de casca dura. Também foi possível verificar a presença de fibras internas na casca dos ovos de iguana que, provavelmente, são responsáveis por conferir a capacidade elástica aos ovos suportando absorção de grandes quantidades de água além do crescimento do embrião. As imagens de ressonância magnética permitiram detalhar o desenvolvimento embrionário sob diferentes condições hídricas, revelando variações na morfologia interna e externa (deformações da casca) do ovo, além de mostrar o impacto de ambientes secos na dinâmica espacial do interior do ovo, resultando em pouco espaço para o desenvolvimento do embrião. Afim de complementar o estudo de imagens, foram realizadas capturas de imagens de ressonância de ovos de *H. mabouia* e *C. latirostris,* onde pudemos observar pela primeira vez a presença de saco de ar em um ovo reptiliano de jacaré de papo amarelo com 90% de incubação, revelando assim mais uma grande semelhança para com as aves, devido sua proximidade evolutiva. Considerando todos os principais dados obtidos, podemos concluir que a disponibilidade de água para o ovo afeta não só suas respostas fisiológicas como também a morfologia da casca, provocando aspecto deformado e reduzindo sua espessura devido à desidratação. Contudo, embriões de iguana apresentaram uma grande tolerância a ambientes hostis, e mesmo sob condições desfavoráveis foram capazes de completar seu desenvolvimento e eclodir.

**Palavras-chave:** Herpetologia, Fisiologia Embrionária, Potencial Hídrico, Osmolalidade, Diagnóstico por Imagem.

**Abstract**

The eggs of flexible hulls are absent from the water reserve provided by the mother, and need to absorb water, necessary for its metabolism, through the incubation medium that, under natural conditions, is directly influenced by the rainfall regime. Thus, with the main objective to understand the embryonic physiology of reptiles that have flexible shell eggs, green iguana eggs were used (Iguana iguana) incubated under different water potentials ranging from extremely dry to extremely wet, which were evaluated parameters reflecting the physiology of water balance of these embryos: osmolality, dry mass, water content and internal pressure of eggs. In addition, diagnostic imaging of scanning electron microscopy and magnetic resonance imaging were also applied to verify the shell morphology of these eggs and the noninvasive evaluation of embryonic development under different water conditions, respectively. Among the main results are a very significant increase of the osmotic concentration of allantoic fluid in extremely dry environment resulting in 385 mOsmol / kg. Mass rates show that embryos exhibit a 158% increase in extremely dry treatments when compared to moist treatments. In dry conditions the eggs showed deformation of the shell and the embryo represented the greater part of the whole egg, with very small fractions of calf and allantois. The thickness of the bark varies according to the water condition, being able to present a reduction of more than 50%. In addition, there is a detrition of the eggshell at the end of incubation resulting mainly from the water action, besides the degradation by microorganisms, and not the mobilization of calcium to the embryo as it occurs in eggs of hard shell. It was also possible to verify the presence of internal fibers in the shell of the iguana eggs, which are probably responsible for imparting the elastic capacity to the eggs, supporting the absorption of large amounts of water besides the growth of the embryo. Magnetic resonance images allowed detail embryonic development under different water conditions, showing changes in internal and external morphology (peeling deformation) of the egg, and show the impact of dry environments in the spatial dynamics of the interior of the egg, resulting in little space for the development of the embryo. In order to complement the study of images were performed resonance imaging catch H. *mabouia* and *C. latirostris* eggs, where we can observe the first time the presence of air bag in a chat yellow reptilian egg alligator 90% of incubation, thus revealing a great similarity to the birds, due to its evolutionary proximity. Considering all the main data obtained, we can conclude that the water availability to the egg affects not only its physiological responses but also the shell morphology, causing deformed appearance and reducing its thickness due to dehydration. However, iguana embryos presented great tolerance to hostile environments, and even under unfavorable conditions were able to complete their development and hatch.

**Key-words:** Herpetology, Embryonic Physiology, Water Potential, Osmolality, Diagnostic Imaging.

**Summary**

# Introduction

## 1. Reptiles

Reptiles are represented in a single Class, Reptilia, Split into four Orders: Chelonia (turles, terrapins and tortoises), Squamata (lizards and snakes), Rynchocephalia (tuataras) and Crocodylia (crocodilians). Oviparity and incubation buried in terrestrial nests are the typical way of reproduction in all Orders; with the exception of some Squamates that exhibt oviparity and curl around the eggs to hatch than (i.e. *Python molurus*) and viviparity (i.e. some snakes), and some species of gecko that leave their eggs exposed (i.e. *Hemidactylus mabouia*) (DEEMING; UNWIN, 2004).

Squamata can be found on all continents except Antarctica. This wide distribution, in tropical, subtropical, arid and even cold regions, shows a wide ecological, physiological and behavioral flexibility, which is corroborated by the great diversity of species, constituting the largest group of living reptiles (ZUG et al., 2001). This distribution occurred, among other factors, due to the cledoic eggs, that allowed the development of the embryo in a diversity of terrestrial environments. However, there are morphological differences between the types of eggs of the various species of reptiles, which may be significant for embryonic development.

## 2. The cleidoic egg

Reptilian eggs are all cleidoic, characterised by large yolks and the development of the embryo to an advanced free-living hatchling. Embryogenesis in the egg is supported by extra-embryonic membranes (Figure 1), i.e. the amnion, chorion, yolk sac and allantoic fluid, that variously offer physical protection, allow utilisation of the yolk, permit respiration, and enable storage of waste products; and they also have a fibrous shell membrane that covering the egg (DEEMING; UNWIN, 2004).

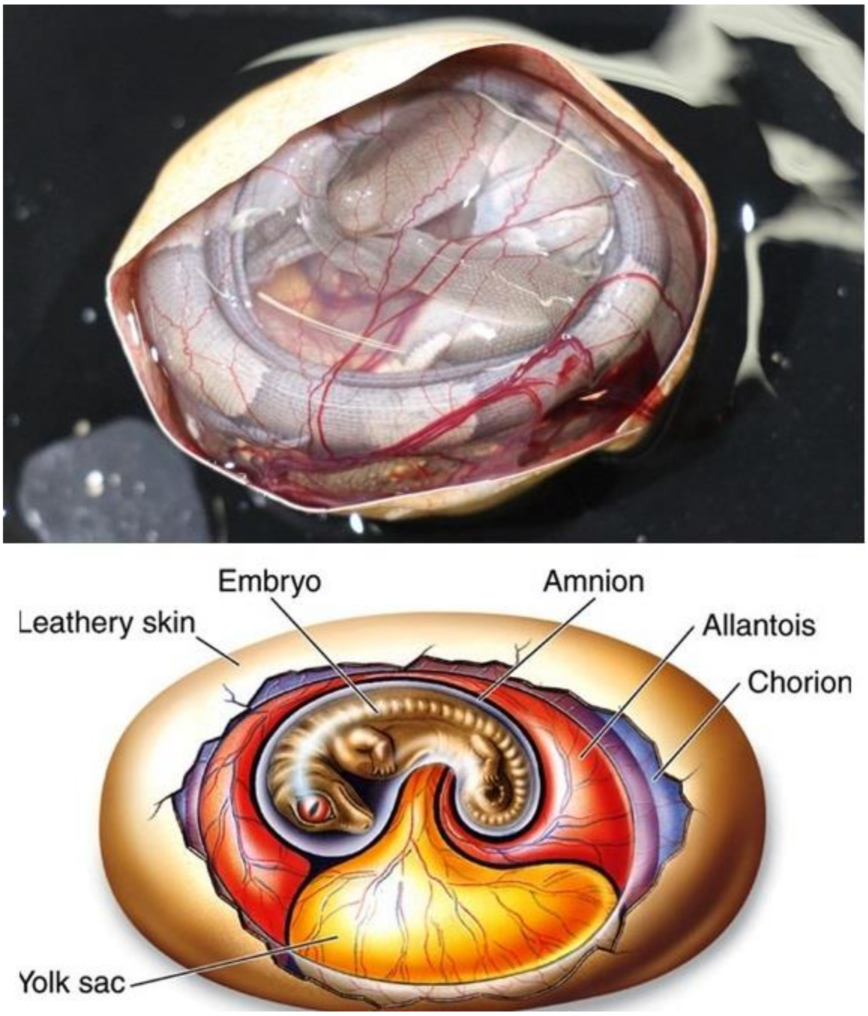



Figure 1. Below: Graphic representation of a reptilian egg and structures (JOVCEV, 2016); Above: Picture of an open iguana egg with 90% of incubation (Personal file).

According to Packard (1982), reptile eggs generally can be divided into three large groups, based on their shell structures: eggs with more flexible shells, those with little or none calcareous layer (most Squamata); eggs with intermediate shell flexibility (some chelonians); and rigid shelled eggs with a well-developed calcareous layer (crocodilians and some chelonians).

In this sense, it is known that eggs of more flexible shells tend to present a greater variability in the gain and loss of water to the environment and may in some cases double or even triple their initial size, from the moment of laying to the final stage of incubation, due to the uptake of water throughout the embryonic development (SARTORI, 2012). For this reason, this type of egg becomes much more susceptible to environmental water variations than the others, with more rigid shells that tend to exchange very little water with the environment, thus presenting little or no change in size during incubation (FEDER et al.,1982; PACKARD et al,1979; M.J. PACKARD et al,1982; WOODALL,1984; MORRIS et al,1983; PACKARD and PACKARD,1988b,1989).

## 3. Embryonic development and the influence of environmental factors

Considering the water susceptibility of eggs with flexible shell and added to the possible environmental adversities that the embryo may face during incubation (periods of drought and / or heavy rainfall), and the absence of parental care characteristic of reptiles (WARNER, 2011), it is necessary that the embryo presents great tolerance to these adversities or that there are physiological modulations that allow their full development. In addition, these conditions may have a profound influence on phenotypic development, thus having important consequences on the physical condition of the animal (WEST-EBERHARD, 2003; GILBERT and EPEL, 2008) and, therefore, survival after hatch.

According to Packard (1981b), approximately 70% of the egg mass of reptiles, at the time that they are layed, is composed of water. The water present in the albumen moves to the yolk sac through an osmotic process of a water potential present in the liquid that is in its interior. This process initiates inside the female and is perpetuated for up to one week after egg laying (PACKARD 1981a). However, the water regulation throughout the incubation process is still unknown. Thus, the water present in the albumen even before the posture could explain why in the first half of the incubation process the eggs show few changes in their mass, both when incubated in drier or wetter environments. This water reserve could prevent water stress in some species of water stress during the first stage of egg incubation. Nevertheless, during the second half of the hatchery period, the reserve will generally have been used making the embryo more vulnerable to environmental conditions and dependent on the uptake of water from the environment, causing a greater variation in egg mass.

Sartori (2012) showed that the green iguana (*Iguana iguana*) eggs accumulate 0.02 mM of urea, representing 82% of the total measurable nitrogen residues accumulated within the allantoic during incubation. That is, it is necessary that the embryo develops properly even with the presence of potentially harmful residues within the egg. For this reason, the study by Tracy (1980) shows that less calcareous eggs are capable of large and rapid absorption of water by the environment, which may be a strategy used to promote the development of embryos, allowing the absorption of water present in the ground.

Eggs incubated on wetter substrates generally tend to have a shorter incubation period, with larger neonates and a higher birth rate than eggs incubated on drier substrates. This shows that these eggs are more sensitive to water variations of the environment (PACKARD, 1982). However, the ideal amount of moisture varies for each species. Still, little is known about the water balance that occurs during embryonic development in reptiles. Although there is robust evidence for the regulation of water and solute inside the eggs of reptiles and birds (BADHAM, 1971; VLECK, 1991), the physiological regulation of water uptake by eggs of these animals is still unclear. The results obtained by Warner (2011) suggest that the water absorption by eggs is, to a significant extent, a passive hydraulic process during the initial incubation; but live embryos are needed to maintain or regulate water uptake in later stages.

The study of the water balance in reptile eggs has not been enough explored, and most of the studies work with rigid shelled eggs (calcareous layer). Thus, the knowledge of the development of more flexible eggs is still unknow. The fact is interesting, since the Squamata, lizards and snakes, which represent the most diversified reptiles and are the ones with the greatest geographical distribution present this type of egg. This study investigates the water relations of flexible shelled eggs in relation to the humidity of the incubation media in lizards. To do so, we used Green Iguana (*Iguana iguana*) eggs, because this animal (Figure 2) lives in different biomes as: Savannah, Semi-arid forest, Pantanal and Amazon rainforest, and therefore they must adjust their physiology according to the different rainfall regimes in order to complete the incubation process.

# Chapter V - General Conclusions

It was first described that vermiculite is a widely used substrate for egg incubation, because it has a high water absorption capacity and also a high temperature retention. However, these two characteristics also turns it a difficult material to measure water potential with good precision.

Many studies that worked with reptile incubation tried to measure the water potential of vermiculite by using, generally, the psychrometer methodology. Nonetheless, it was explained in Chapter I that this is not the best choice of methodology for vermiculite. And despite we tried not only psychrometer, buy tension table and Richard's Chamber, it was Gypsum Block the methodology that provide good results in a short period of time.

This means that to use data from papers that didn't used Gypsum Block to measure water potential in incubation media it's, probably, recommended an application of a correction factor or a new measurement of these data with the appropriated methodology.

The different treatments of water potential of vermiculite used in this study revealed some physiological responses in *Iguana iguana* eggs described in Chapter II. In this section, we firstly see that the embryo represents a fraction of 52.15% of the egg in ED condition, meaning an increase of 158% if compared to the embryo fraction of EW condition. This didn't mean an increase of embryo size in ED, but infers that eggs incubated in extremely dry conditions doesn't have high amounts of allantoic fluid (29.97% in ED; 48.65% in EW) or yolk (12.71% in ED; 21.43% in EW) when compared to eggs incubated in extremely wet conditions. Therefore, the embryo occupies more space inside the egg.

Also in Chapter II, the osmotic parameters seemed altered in ED conditions with the higher value of 385 mOsmol/kg in allantoic fluid at 90% IP against to 47 mOsmol/kg at 90% IP in EW condition. And besides the ED condition, all other treatments seem to decrease the osmotic concentration during IP. Yolk osmotic values also showed a pattern to decrease along the IP in all treatments, with statistical significant difference in ED and D conditions.

It seems that the first third of IP is a critical period, since generally *I. iguana* present higher allantoic osmotic values at this point then in the rest of incubation. This could also be a strategy for water absorption, since the egg is more concentrated at 30% IP it's easier to absorb water from the external environment. However, more study must be done in this area in order to prove this.

Just laid eggs have an amount of water of 71% from the total initial egg mass. Eggs in ED and VD conditions present the lower dry mass, showing statistical difference from the other treatments. However, the hatchlings from these treatments didn't differed in weight and length from the others.  This may indicate that the lack of water didn't affect the size of hatchling at first, but it will interfere in physiological parameters such as osmolality that could be damaging for the embryo if exposed in extremely dry conditions. To prove that, more study must be done in this field, probably by measuring allantoic osmolality of *I. iguana* eggs incubated in dryer conditions than the treatments used in this work.

Because of this water impacts on the physiology of the embryo, Chapter III is dedicated to investigate images from scanning electron microscopy of *Iguana iguana* eggshell under different water potentials. Those images showed that I. iguana eggshells present pores in a very spaced pattern from one to another, making it difficult to observe them by area, and not allowing an estimate of the total pore number present in the shell. Exactly because this pore-spaced pattern, we can suggest that water and gas exchanges should be made mostly through the permeability of the eggshell itself than only by the pores.

Also, in Chapter III we could see the effect of the water and its lack in eggshells at 90% of IP. At this point, we presented a wild morphological variation for the external part of the eggshell and also a significant thickness reduction in ED condition (52% of reduction), when compared to the initial thickness values at the moment of laying, probably due to dehydration. And finally, we described the inner layer of the shell that is constituted by a network of organic fibers that provide the flexibility capacity, which is an outstanding characteristic of this type of egg.

In order to understand the affect of water in the morphology of the egg, we also did RMI scans of *I.iguana* eggs under different water potentials and at different incubation periods. These images were presented in Chapter IV, revealing that at 50% of incubation under ED condition the egg is deformed and it was impossible to detect the presence of allantoic fluid. However, at 90% of incubation under ED condition the egg doesn't seemed deformed, but still the allantoic fluid wasn't located.

In comparison of the other substrate hydratation conditions was possible to see a difference in the yolk, where in the Dry condition it is much smaller than in the Intermediate and Wet conditions used. This is probably due to the dehydration but may also be because of the production of metabolic water in wetter conditions.

Chapter IV is also dedicated to the morphological study of rigid shelled eggs as *C. latirostris* and *H. mabouia*, that were scanned at 90% of incubation but in this case the water potential wasn't the main goal because we wanted to see the morphology of the embryo, the structures of the egg, and if the MRI technique is valid for rigid shelled eggs due to the high thickness of the eggshell.

MRI was valid for this kind of egg, and we also discovered some remarkable thing about *C. latirostris* egg. This caiman egg presents an air sac in front of the nose of the embryo, just like avian eggs, and *H. mabouia* doesn't has this structure proving that it's not related to rigid shelled eggs.

The air sac is used in "*pipping*" behavior, showing that this animal is very similar to birds due to its evolutionary proximity. Other possible use for the air sac is as an acoustic box, since both crocodilians and birds vocalize inside the egg when it's close to hath, in order to synchronize the hatchling.

However, more studies must be done in this area. Firstly, by scanning more eggs from more crocodilian species to see if this structure is present in all Order Crocodylia. Secondly, by investigate if there is any relation of the air sac to the vocalization pre-hatchling as an acoustic box.

In summary, this work provides some insights about the influence of the water during incubation of flexible shelled eggs of *Iguana iguana*, revealing that these eggs can resist to hostile environments that lack in water and still hatch. However, there is still a lot to investigate about dry substrate tolerance in embryo physiology and shell morphology due to flexibility. In addition, this study opens a new field of work in crocodilians eggs with the aim to see if air sac is truly present in all Order Crocodylia, and if there is other function for it than "*pipping*".

# References

**References Introduction**

BADHAM, J.A. Albumen formation in eggs of the *agamid Amphibolurus barbatus barbatus*. **Copeia** 1971, 543–545. 1971.

DEEMING, D.C.; UNWIN, D.M. Reptilian incubation: evolution and the fossil record. In: DEEMING, D.c.. **Reptilian Incubation:** Environment, Evolution and Behaviour. [s.l]: Nottingham University Press, 2004. p. 1-14.

FEDER, M.E.; SATEL, S.L.; GIBBS, A.G. Resistance of the Shell membrane and mineral layer to diffusion of oxigen and water inflexibles shelled eggs of the snapping turtle (*Chelydra serpentina*). **Respir. Physiol**. 49:279-291. 1982.

GILBERT, S.F.; EPEL, D. Ecological Developmental Biology: **Integrating Epigenetics, Medicine, and Evolution**. Sinauer, Sunderland. 2008.

JOVCEV, D. **Gradja amniotskog jajeta.** 2016. Disponível em: <https://www.thinglink. com/scene/838062256355803136>. Acesso em: 17 jul. 2018.

MORRIS, K.A.; PACKARD, G.C.; BOARDMAN, T.J; PAUKSTIS, G.L.; PACKARD, M.J. Effect of the hydric environment on growth of embryonic snapping turtles (*Chelydra serpentina*). **Herpetologica**. 39:272-285. 1983.

PACKARD, G.C.; TAIGEN, T.L.; PACKARD, M.J.; SHUMAN, R.D. Water-vapor conductance of testudinian and crocodilian eggs (classReptilia). **Respir.Physiol**.38:1-10. 1979.

PACKARD, G.C.; PACKARD, M.J.; BOARDMAN, T.J.; ASHEN, M. D. Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles. **Science.** [s.l.], p. 471-473. 22 abr. 1981a.

PACKARD, G. C.; PACKARD, M.J.; BOARDMAN, T.J. Patterns and Possible Significance of Water Exchange by Flexible-Shelled Eggs of Painted Turtles (Chrysemys picta). **Physiological Zoology.** [s.l.], p. 165-178. jan. 1981b.

PACKARD, M.J.; PACKARD, G.C.; BOARDMAN, T.J. Structure of Eggshells and Water Relations of Reptilian Eggs. **Herpetologica.** [s.l.], p. 136-155. mar. 1982.

PACKARD, G.C.; PACKARD, M.J. Water relations of embryonic snapping turtles (Chelydra serpentina)exposed to wet or dry environments at different times in incubation. **Physiol.Zool.**61:95-106. 1988b.

PACKARD,G.C.; PACKARD, M.J. Control of metabolism and growth in embryonic turtles: A test of the urea hypothesis. **J.Exp.Biol.**147:203-216. 1989.

SARTORI, M.R.; TAYLOR, E.W.; ABE, A.S.. Nitrogen excretion during embryonic development of the green iguana, Iguana iguana (Reptilia; Squamata). **Comparative Biochemistry And Physiology, Part A.** [s. L.], p. 210-214. 08 jun. 2012.

TRACY, C. R. Water Relations of Parchment-Shelled Lizard (Sceloporus undulatus) Eggs. **Copeia,** [s. L.], v. 1980, n. 3, p.478-482, 6 set. 1980. Disponível em: <http://www.jstor.org/stable/1444525>. Acesso em: 13 dez. 2013.

VLECK, D. Water economy and solute regulation of reptilian and avian embryos. In: Deeming, D.C., Ferguson, M.W.J. (Eds.), Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. **Cambridge University Press**, Cambridge, pp. 245–259. 1991.

WARNER, D.A.; MOODY, M.A.; TELEMECO, R.S. Is water uptake by reptilian eggs regulated by physiological processes of embryos or a passive hydraulic response to developmental environments? **Comparative Biochemistry And Physiology, Part A.** [s. L.], p. 421-425. 06 jul. 2011.

WEST-EBERHARD, M.J. Developmental Plasticity and Evolution. **Oxford University Press**, Oxford. 2003.

WOODALL, P.F. The structure and some functional aspects of the eggshell of broad-shelled river tortoise Chelodina expansa (Testudinata: Chelidae). **Aust. J. Zool.** 32:7-14. 1984.

ZUG, G.R.; VITT, L.J.; CALDWELL, J.P. Herpetology-An Introductory Biology of Amphibians and Repitiles. **San Diego: Academic Press** (Second Edition), 2001. 630p.

**References Chapter I**

ALMEIDA, A.P.S.; ARAÚJO, F.S.; SOUZA, G.S. de. Determinação da curva parcial de retenção de água de um latossolo vermelho por tensiomêtria. **Scientia Plena,** [s.l.], v. 6, n. 9, p.1-5, 2010.

ANTINORO, C.; BAGARELLO, V.; FERRO, V.; GIORDANO, G.; IOVINO, M. A simplified approach to estimate water retention for Sicilian soils by the Arya-Paris model. **Geoderma**, Amsterdam, v. 213, p. 226-234, 2014.

BITTELLI, M.; FLURY, M. Errors in water retention curves determined by pressure plates. **SSSA Journal**, Madison, v.73, p.1453-1460, 2009.

BOOTH, D. T.; THOMPSON, M. B.; HERRING, S. How incubation temperature influences the physiology and growth of embryonic lizards. **Journal Of Comparative Physiology B**: Biochemical, Systemic, and Environmental Physiology, [s.l.], v. 170, n. 4, p.269-276, 16 jun. 2000. Springer Nature. http://dx.doi.org/10.1007/s003600000097.

BOOTH, D.T. Incubation of rigid-shelled turtle eggs: do hydric conditions matter? **J. Comp. Phsyiol. B,** v. 172, p. 627–633. 2002.

BROWN, G.P., SHINE, R. Effects of nest temperature and moisture on phenotypic traits of hatchling snakes (*Tropidonophis mairii*, Colubridae) from tropical Australia. **Biol. J. Linn. Soc**. v. 89, p. 159–168. 2006.

CASSEL, D. K.; KLUTE, A. Water potential: Tensiometry. In: CASSEL, D. K.. **Physical and Mineralogical Methods.** North Carolina: American Society of Agronomy, 1986. p. 563.

CAMPBELL, G.S. and W.H. GARDNER, 1971. Psychrometric measurement of soil water potential: temperature and bulk density effects. **Soil Sci. Soc. Am. Proc.** 35: 8-12.

COSTA, W.A.; OLIVEIRA, C. A. S.; KATO, E. Modelo de ajuste e métodos para determinação da curva de retenção de água de um Latossolo Vermelho Amarelo. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v.32, n.2, p.515-523, 2008.

DU, W.G. Water exchange of flexible-shelled eggs and its effect on hatchling traits in the Chinese skink *Eumeces chinensis*. J. **Comp. Phsyiol. B**, v.174, p.489–493. 2004.

DU, W.G; SHINE, R. The influence of hydric environments during egg incubation on embryonic heart rates and offspring phenotypes in a scincid lizard (*Lampropholis guichenoti*). **Comparative Biochemistry And Physiology Part A**: Molecular & Integrative Physiology, [s.l.], v. 151, n. 1, p.102-107, set. 2008. Elsevier BV. http://dx.doi.org/10.1016/j.cbpa.2008.06.005.

DU, W.G.; WANG, L.; SHEN, J.W. Optimal temperatures for egg incubation in two Geoemydid turtles: Ocadia sinensis and Mauremys mutica. **Aquaculture**, [s.l.], v. 305, n. 1-4, p.138-142, jul. 2010. Elsevier BV. http://dx.doi.org/10.1016/j.aquaculture.2010.03.032.

DU, W.G., ZHENG, R.Q. Egg survival and hatchling traits of the Chinese threekeeled pond turtle (*Chinemys reevesii*) incubated in different hydric environments. **Acta Zool. Sin.** v. 50, p. 132–135. 2004.

DULEY, F. L.; KELLY, L. L. Effect of Soil Type, Slope, and Surface Conditions on Intake of Wate. **Historical Research Bulletins Of The Nebraska Agricultural Experiment Station**, [s.l.], v. 66, p.1913-1993, 1939.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Centro Nacional de Pesquisas de Solos. **Manual de métodos de análises de solo.** 2.ed. Brasília, Produção de Informação, 1997. 212p.

FELICIANO, J. J. S. **Métodos alternativos para obtenção da curva de retenção da água no solo.** 2005. 103f. Dissertação (Mestrado em Engenharia Agrícola) - Universidade Estadual do Oeste do Paraná, Cascavel, 2005.

FREITAS JUNIOR, E.; SILVA, E.M. Uso da centrífuga para determinação da curva de retenção de água do solo, em uma única operação. **Pesquisa Agropecuária Brasileira,** Brasília, v.19, p. 1423-1428, 1984.

GOMBOS, M. The Impact of Clay Minerals on Soil Hydrological Processes. In: Gomboš, Milan. **Clay Minerals in Nature – Their Characterization, Modification and Application.** Czech Republic: Intech, 2012. Cap. 07. p. 119-148.

GRIGOLON, G. B. **Curva de retenção de água no solo determinada a partir de um número mínimo de pares de umidade e tensão na câmara de Richards.** 2013. 82 f. Dissertação (Mestrado) - Curso de Mestrado em Ciências, Universidade de São Paulo, Piracicaba, 2013.

GUBIAN, P.I.; REICHERT, J.M.; CAMPBELL, C.; REINERT, D.J.; GELAIN, N.S. Assessing erros and accuracy in dew-point potentiometer and pressure plate extractor measurements. **SSSA Journal**, Madison, v.77, n.1, p.19-24, 2012.

GUTZKE, W.H.N.; PACKARD, G.C. Influence of the Hydric and Thermal Environments on Eggs and Hatchlings of Bull Snakes *Pituophis melanoleucus*. **Physiological Zoology**, [s.l.], v. 60, n. 01, p.9-17, fev. 1987a.

GUTZKE, W.H.N.; PACKARD, G.C.; PACKARD, M.J.; BOARDMAN T.J. Influence of the Hydric and Thermal Environments on Eggs and Hatchlings of PaintedTurtles (*Chrysemys picta*). **Herpetologica**, [s.l.], v. 43, n. 04, p.393-404, dez. 1987b.

HALLIKAINEN, M.; ULABY, F.T.; DOBSON, M.C.; EL-RAYES, M.A.; WU, L.K. Microwave Dielectric Behavior of Wet Soil-Part 1: Empirical Models and Experimental Observations. **Ieee Transactions On Geoscience And Remote Sensing**, [s.l.], v. -23, n. 1, p.25-34, jan. 1985. Institute of Electrical and Electronics Engineers (IEEE). http://dx.doi.org/10.1109/tgrs.1985.289497.

HONG, LI.; ZONG-SHI, ZHOU.; TING, WU.; YAN-QING, WU.; XIANG, JI. Do fluctuations in incubation temperature affect hatchling quality in the Chinese soft-shelled turtle *Pelodiscus sinensis*? **Aquaculture,** [s.l.], v. 2013, n. 407, p.91-96, 10 maio 2013.

JANZEN, F.J.; WILSON, M.E.; TUCKER, J.K.; FORD, S.P. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. **General and Comparative Endocrinology**, [s.l.], v. 111, n. 3, p.306-317, set. 1998. Elsevier BV. http://dx.doi.org/10.1006/gcen.1998.7115.

JAY-ALLEMAND, C.; CAPELLI, P.; CORNU, D. Root development of in vitro hybrid walnut microcuttings in a vermiculite containing gelrite medium. **Scientia Horticulturae,** [s.l.], v. 1992, n. 51, p.335-342, jan. 1992.

KOOPMANS, R.W.R.; MILLER, R.D. Soil Freezing and Soil Water Characteristic Curves. **Soil Science Society of America Journal**, [s.l.], v. 30, n. 6, p.680-685, 1966. Soil Science Society of America. http://dx.doi.org/10.2136/sssaj1966.03615995003000060011x.

MARCOS, C., ARANGO, Y.C., RODRIGEZ, I. X-ray diffraction studies of the thermal behaviour of commercial vermiculites. **Appl. Clay Sci**. 42: 368-378. 2009.

MATHIESON, A.M., WALKER, G.F. Crystal Structure of Magnesium–Vermiculite. **Am. Miner**. 39: 231–255. 1954.

MBONIMPA M.; AUBERTIN M., ASCE M.; MAQSOUD A.; and BUSSIÈRE B. Predictive Model for the Water Retention Curve of Deformable Clayey Soils. **Journal of Geotechnical and Geoenvironmental Engineering**. Vol. 132, Issue 9. 2006.

MEDEIROS, S.D. **Determinação da curva de retenção da água no solo através do fatiamento de amostras centrifugadas**. 1987. 66f. Dissertação (Mestrado em Agronomia) - Universidade de São Paulo, Piracicaba, 1987.

MELO FILHO, J. F. de; SACRAMENTO, J. A. A. S. do; CONCEIÇÃO, B. P. S. Curva de retenção de água elaborada pelo método do psicrômetro para uso na determinação do índice "S" de qualidade física do solo. **Engenharia Agrícola,** [s.l.], v. 35, n. 5, p.959-966, out. 2015. FapUNIFESP (SciELO). http://dx.doi.org/10.1590/1809-4430-eng.agric.v35n5p959-966/2015.

MOREIRA, W. H.; PETEAN, L. P.; JUNIOR, E. B.; TORMENA, C. A.; FIGUEIREDO, G. C.; Silva, A.P. Método alternativo para quantificação do intervalo hídrico ótimo em laboratório. **Revista Brasileira de Ciência do Solo,** [s. l.], v. 38, n. 1, p.158-165, 2014.

MORRIS, K. A.; PACKARD, G. C.; BOARDMAN, T. J.; Paukstis, G. L.; Packard, M. J. Effect of the Hydric Environment on Growth of Embryonic Snapping Turtles (*Chelydra serpentina*). **Herpetologica**, [s.l.], v. 39, n. 3, p.272-285, set. 1983.

OFTEDAL, O. T. The origin of lactation as a water source for parchment-shelled eggs. **Journal of Mammary Gland Biology and Neoplasia**, [s.l.], v. 7, n. 3, p.253-266, 2002. Springer Nature. http://dx.doi.org/10.1023/a:1022848632125.

PACKARD, G. C.; TAIGEN, T. L.; BOARDMAN, T. J.; PACKARD, M.J.; TRACY, C.R. Changes in mass of softshell turtle (*Trionyx spiniferus*) eggs incubated on substrates differing in water potential. **Herpetologica**, [s.l.], v. 35, n. 1, p.78-86, mar. 1979.

PACKARD, G. C.; PACKARD, M. J.; BOARDMAN, T. J. Patterns and Possible Significance of Water Exchange by Flexible-Shelled Eggs of Painted Turtles (*Chrysemys picta*). **Physiological Zoology**, [s.l.], v. 54, n. 1, p.165-178, jan. 1981a. University of Chicago Press. http://dx.doi.org/10.1086/physzool.54.1.30155815.

PACKARD, G. C. et al. Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles. **Science**, [s.l.], v. 213, n. 4506, p.471-473, 24 jul. 1981b. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.213.4506.471.

PACKARD, Mary J.; PACKARD, Gary C.; BOARDMAN, Thomas J. Structure of Eggshells and Water Relations of Reptilian Eggs. **Herpetologica**, [s.l.], v. 38, n. 01, p.136-155, mar. 1982.

PACKARD, G. C.; PACKARD, M. J. Patterns of Nitrogen Excretion by Embryonic Softshell Turtles (*Trionyx spiniferus*) Developing in Cleidoic Eggs. **Science**, [s.l.], v. 221, n. 4615, p.1049-1050, 9 set. 1983. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.221.4615.1049.

PACKARD, G. C.; PACKARD, M. J.; GUTZKE, W. H. N. Influence of Hydration of the Environment on Eggs and Embryos of the Terrestrial Turtle *Terrapene ornata*. **Physiological Zoology**, [s.l.], v. 58, n. 5, p.564-575, set. 1985. University of Chicago Press. http://dx.doi.org/10.1086/physzool.58.5.30158583.

PACKARD, G.C.; PACKARD, M.J.; MILLER, K. and BOARDMAN, T.J. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. **Ecology**, v. 68, n. 4, p.983-993, 1987.

PACKARD, M. J. Sources of Mineral for Green Iguanas (*Iguana iguana*) Developing in Eggs Exposed to Different Hydric Environments. **Copeia,** [s.l.], v. 1992, n. 3, p.851-858, ago. 1992.

PACKARD, G.C. Water relations of chelonian eggs and embryos: is wetter better? **Am. Zool**, [s.l.], v. 39, p.289–303. 1999.

PEREIRA, H. C. A cylindrical gypsum block for moisture studies in deep soils. **Journal of Soil Science,** [s.l.], v. 02, n. 02, p.212-223, jan. 1951.

SCANLON, BRIDGET R., BRIAN J. ANDRASKI, and JIM BILSKIE. "3.2. 4 Miscellaneous methods for measuring matric or water potential." **Methods of Soil Analysis: Part 3 The soil solution phase**, [s.l.], p. 643-670. 2002.

SEKI, K. SWRC fit - a nonlinear fitting program with a water retention curve for soils having unimodal and bimodal pore structure. **Hydrol. Earth Syst. Sci. Discuss**., 4: 407-437. doi:10.5194/hessd-4-407-2007.

SHINZATO, M. C. **Estudo experimental de retenção de íons metálicos em vermiculita.** 1995. 93 f. Dissertação (Mestrado) - Curso de Mineralogia e Petrologia, Instituto de Geociências, Universidade de São Paulo, São Paulo, 1995.

SIFUENTES-ROMERO, I., TEZAK, B.M., MILTON, S.L. and WYNEKEN, J. Hydric environmental effects on turtle development and sex ratio. **Zoology,** 126, 89-97, 2018.

SPAANS, E. J.; BAKER, J. M. Calibration of Watermark soil moisture sensors for soil matric potential and temperature. **Plant And Soil,** [s.l.], v. 1, n. 143, p.213-217, fev. 1992.

TAVARES, M.H.F.; FELICIANO, J.J.S.; VAZ, C.M.P. Análise comparativa de métodos para determinação da curva de retenção de água em solos. **Irriga**, Botucatu, v.13, n.4, p.517-524, 2008.

TRACYC, R., G. C. PACKARD AND M. J. PACKARD. Water relations of chelonian eggs. **Physiol. Zool**. v. 51, p.378-387. 1978.

TRACY, C. RICHARD. Water Relations of Parchment-shelled Lizard (*Sceloporus undulatus*) Eggs. **Copeia,** [s.l.], v. 1980, n. 03, p.478-482, 06 set. 1980.

THOMPSON, M. B.; SPEAKE, B. K.; RUSSELL, K. J.; McCARTNEY, R. J.; SURAI, P.F. Changes in fatty acid profiles and in protein, ion and energy contents of eggs of the Murray short-necked turtle, *Emydura macquarii* (Chelonia, Pleurodira) during development. **Comparative Biochemistry and Physiology Part A**: Molecular & Integrative Physiology, [s.l.], v. 122, n. 1, p.75-84, jan. 1999. Elsevier BV. http://dx.doi.org/10.1016/s1095-6433(98)10150-2.

VALÁŁKOVÁ, M.; MARTYNKOVÁ, G. S. Vermiculite: Structural Properties and Examples of the Use. In: VALASKOVA, Marta. **Clay Minerals in Nature – Their Characterization, Modification and Application.** Czech Republic: Intech, 2012. Cap. 11. p. 209-238.

VAN DYKE, J. U.; PLUMMER, M. V.; BEAUPRE, S. J.. Residual yolk energetics and postnatal shell growth in Smooth Softshell Turtles, *Apalone mutica*. **Comparative Biochemistry And Physiology Part A**: Molecular & Integrative Physiology, [s.l.], v. 158, n. 1, p.37-46, jan. 2011. Elsevier BV. http://dx.doi.org/10.1016/j.cbpa.2010.08.026.

WARNER, D. A.; MOODY, M. A.; TELEMECO, R. S. Is water uptake by reptilian eggs regulated by physiological processes of embryos or a passive hydraulic response to developmental environments? **Comparative Biochemistry And Physiology Part A**: Molecular & Integrative Physiology, [s.l.], v. 160, n. 3, p.421-425, nov. 2011. Elsevier BV. http://dx.doi.org/10.1016/j.cbpa.2011.07.013.

WILSON, GCS. Use of vermiculite as a growth medium for tomatoes. **Acta Horticulturae** 150: 283-288. 1983.

Xiang, JI.; BRAÑA, F. The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). **Comparative Biochemistry And Physiology Part A**: Molecular & Integrative Physiology, [s.l.], v. 124, n. 2, p.205-213, out. 1999. Elsevier BV. http://dx.doi.org/10.1016/s1095-6433(99)00111-7.

XIANG, JI.; WEI-GUO, DU. The effects of thermal and hydric environments on hatching success, embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata*. **Comparative Biochemistry and Physiology,** [s.l.], v. 2001, n. 129, p.461-471, 31 dez. 2000.

ZHAO, B.; CHEN, Y.; WANG, Y.; DING, P.; Du, W. G. Does the hydric environment affect the incubation of small rigid-shelled turtle eggs? **Comparative Biochemistry And Physiology,** [s.l.], v. 2013, n. 164, p.66-70, 24 set. 2012.

**References Chapter II**

Baldwin. E., 1964. An Introduction to Comparative Biochemistry. **Cambridge Univ. Press**. Cambridge. ed. 4.

Cassel, D. K.; Klute., 1986. A. Water potential: Tensiometry. In: CASSEL, D. K.. **Physical and Mineralogical Methods.** North Carolina: American Society of Agronomy,. p. 563.

Clegg, J. S., 1981. Metabolic consequences of the extent and disposition of the aqueous intracellular environment. **J. Exp. Zool.** 215, 303-313.

Feder M.E.; Satel S.L., 1982. Gibbs A.G. Resistance of the Shell membrane and mineral layer to diffusion of oxigen and water inflexibles shelled eggs of the snapping turtle (*Chelydra serpentina*). **Respir. Physiol**. 49, 279-291.

Grim, R. E., 1968. **Clay mineralogy**. Second edition. McGraw- Hill, New York, New York, USA.

Lynn, W. G., and T. Von Brand., 1945. Studies on the oxygen consumption and water metabolism of turtle embryos. **Biol. Bull**. 88, 112-125.

Needham, J., 1931. Characterized the cleidoic egg as "a closed box, with walls which can only be penetrated by matter in the gaseous state”. **Chemical Embryology**, Cambridge Univ. Press, Cambridge, 16151.

Morris K.A.; Packard G.C.; Boardman T.J; Paukstis G.L.; Packard M.J., 1983. Effect of the hydric environment on growth of embryonic snapping turtles (*Chelydra serpentina*). **Herpetologica**. 39, 272-285.

Tracy, C. R., G. C. Packard, and M. J. Packard., 1978. Water relations of chelonian eggs. **Physiol. Zool**. 51, 378-387.

Packard, Gary C. et al., 1979. Changes in Mass of Softshell Turtle (*Trionyx spiniferus*) Eggs Incubated on Substrates Differing in Water Potential. **Herpetologica,** 35, 78-86.

Packard, Gary C. et al., 1981a. Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles. **Science. 22,** 471-473.

Packard, Gary C.; Packard, Mary J.; Boardman, Thomas J., 1981b. Patterns and Possible Significance of Water Exchange by Flexible-Shelled Eggs of Painted Turtles (Chrysemys picta). **Physiological Zoology.** 165-178.

Packard, Mary J.; Packard, Gary C.; Boardman, Thomas J., 1982a. STRUCTURE OF EGGSHELLS AND WATER RELATIONS OF REPTILIAN EGGS. **Herpetologica,** 38, 136-155.

Packard, Gary C.; Packard, Mary J., 1982b. Patterns of Nitrogen Excretion by Embryonic Softsheli Turtles (Trionyx spiniferus) Developing in Cleidoic Eggs. **Science**, 221, 1049-1050.

Packard, Gary C. et al., 1983. Biology Influence of Water Exchanges by Flexible-Shelled Eggs of Painted Turtles *Chrysemys picta* on Metabolism and Growth of Embryos. **Physiological Zoology**, 217-230.

Packard, G. C. and M. J. Packard., 1984. Effects of the hydric environment on metabolism of embryonic snapping turtles do not result from altered patterns of sexual differentia-tion. **Copeia** 1984, 547-550.

Packard, Gary C.; Packard, Mary J.; Gutzke, William H. N., 1985. Influence of Hydration of the Environment on Eggs and Embryos of the Terrestrial Turtle Terrapene ornata. **Physiological Zoology,** 58, 564-575.

Packard, Mary J.; Phillips, John A.; Packard, Gary C., 1992. Sources of Mineral for Green Iguanas (*Iguana iguana*) Developing in Eggs Exposed to Different Hydric Environments. **Copeia,** 1992, 851-858.

Rand, A. S. Iguana Egg Mortality within the Nest. **Copeia**, [s.l.], v. 1980, n. 3, p.531-534, 06 set. 1980.

Ratterman, R. J.; Ackerman, A. R. The Water Exchange and Hydric Microclimate of Painted Turtle (Chrysemys psicta) Eggs Incubatinign Field Nests. **Physiological Zoology**, [s.l.], v. 62, n. 5, p.1059-1079, 1989.

Sartori, M.R.; Taylor, E.W.; Abe, A.S., 2012. Nitrogen excretion during embryonic development of the green iguana, *Iguana iguana* (Reptilia; Squamata). **Comparative Biochemistry And Physiology, Part A,** 210-214.

Seymour, R. S.; Ackerman, R. A. Adaptations to Underground Nesting in Birds and Reptiles. **Amer. Zool**, [s.l.], v. 20, p.437-447, 1980.

Warner, Daniel A.; Moody, Melissa A.; Telemeco, Rory S., 2011. Is water uptake by reptilian eggs regulated by physiological processes of embryos or a passive hydraulic response to developmental environments? **Comparative Biochemistry And Physiology, Part A,** 421-425.

William H. N. G et al., 1987. INFLUENCE OF THE HYDRIC AND THERMAL ENVIRONMENTS ON EGGS AND HATCHLINGS OF PAINTED TURTLES (CHRYSEMYS PICTA). **Herpetologica**, 43, 393-404.

Woodall P.F., 1984. The structure and some functional aspects of the eggshell of broad-shelled river tortoise Chelodina expansa (Testudinata: Chelidae). **Aust. J. Zool.** 32,7-14.

## References Chapter III

BELINSKY, A. et al. Water in Reptilian Eggs and Hatchlings *In:* Deeming, D. C. (ed.), **Reptilian Incubation. Environment, Evolution and Behaviour**. Nottingham, U.K: Nottingham University Press, 2004.

BAIRD, T., AND S. E. SOLOMON. Calcite and aragonite in the egg shell of *Chelonia mydas* **L. J. Exp. Mar. Biol. Ecol**. 36:295-303. 1979.

CHANG, YIN; CHEN, PO-YU. Hierarchical structure and mechanical properties of snake (*Naja atra*) and turtle (*Ocadia sinensis*) eggshells. **Acta Biomaterialia**, [s.l.], v. 31, p.33-49, fev. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.actbio.2015.11.040.

ELAROUSSI, M. A., L. R. FORTE,H . V. BIELLIER,S. L. EBER,R . E. POELLING, AND W. J. KRAUSE. Indexes of vitamin D deficiency in Japanese quail embryos. **Am. J. Physiol**. 254:E639-E651. 1988.

ERBEN, H. K. Ultrastrukturen und Mineralisation rezenter und fossiler Eischalen bei Vogeln und Reptilien. **Biomineralisation** 1:1-66. 1970.

ERBEN, H. K., AND H. NEWESELY. Kristalline Bausteine und Mineralbestand von Kalkigen Eischalen. **Biomineralisation** 6:32-48. 1972.

FERGUSON, M. W. J. The structure and or-ganization of the eggshell and embryonic membranes of *Alligator mississippiensis*. **Trans. Zool. Soc**. London.1982.

KRAMPITZ, G., H. K. ERBEN, AND K. KRIESTEN. Uber Aminosaurenzusammensetzung und Struktur von Eischalen. **Biomineralisation** 4:87-99.1972.

KRIESTEN, K. Untersuchungen uber Ultrastruktur, Proteinmuster und Aminosaurenzusam-mensetzung der Eischalen von Testudo elephantopus, *Caiman crocodilus* and *Iguana iguana*. **Zool. Jb. Anat**. 94:101-122. 1975.

NUNES, RENATA BRANDT. EVOLUÇÃO DA VIVIPARIDADE EM SQUAMATA: CENÁRIOS EVOLUTIVOS E RARIDADE DOS ESTÁGIOS INTERMEDIÁRIOS. **Revista da Biologia**, [s.l.], v. 01, n. 0, p.21-26, 04 nov. 2008.

PACKARD, M. J., AND G. C. PACKARD. Structure of the shell and tertiary membranes of eggs of softshell turtles (*Tronyx spiniferus*). **J. Mor-phol.** 159:131-143. 1979a.

PACKARD, G. C. et al. WATER-VAPOR CONDUCTANCE OF TESTUDINIAN AND CROCODILIAN EGGS (CLASS REPTILIA). **Respiration Physiology**, [s.l.], v. 38, p.01-10, 1979b.

PACKARD, M. J. Ultrastructural morphology of the shell and shell membrane of eggs of common snapping turtles (*Chelydra serpentina*). **J. Morphol.** 165:187-204. 1980.

PACKARD, G. C. et al. PATTERNS AND POSSIBLE SIGNIFICANCE OF WATER EXCHANGE BY FLEXIBLE-SHELLED EGGS OF PAINTED TURTLES (CHRYSEMYS PICTA). **Physiological Zoology**, [s.l.], v. 54, n. 01, p.165-178, jan. 1981.

PACKARD, M. J., L. K. BURNS, K. F. HIRSCH, AND G. C. PACKARD. Structure of eggshells of *Callisaurus draconoides* (Reptilia, Squamata, Iguanidae). **Zool. J. Linn. Soc.**, in press. 1982a.

PACKARD, MARY J.; PACKARD, GARY C.; BOARDMAN, Thomas J.. Structure of Eggshells and Water Relations of Reptilian Eggs. **Herpetologica**, [s.l.], v. 38, n. 01, p.136-155, mar. 1982b.

PACKARD, M.J., and G. C. PACKARD. Patterns of mobilization of calcium, magnesium, and phosphorus by embryonic yellow headed blackbirds (*Xanthocephalus xanthocephalus*). **J. Comp. Physiol**. B 160:649-654. 1991.

PACKARD, MARY J.; PHILLIPS, JOHN A.; PACKARD, GARY C.. Sources of Mineral for Green Iguanas (*Iguana iguana*) Developing in Eggs Exposed to Different Hydric Environments. **Copeia**, [s.l.], v. 1992, n. 03, p.851-858, 18 ago. 1992.

PACKARD, GARY C.. Water Relations of Chelonian Eggs and Embryos: Is Wetter Better? **Amer. Zool**, [s.l.], v. 39, n. 02, p.289-303, 1999.

RAND, A. S. Desiccation Rates in Crocodile and Iguana Eggs. **Herpetologica**, [s.l.], v. 24, n. 2, p.178-180, jun. 1968.

SARTORI, M.R.; TAYLOR, E.W.; ABE, A.S.. Nitrogen excretion during embryonic development of the green iguana, *Iguana iguana* (Reptilia; Squamata). **Comparative Biochemistry And Physiology, Part A.** [s. L.], p. 210-214. 08 jun. 2012.

SEXTON, OWEN J. et al. Eggshell Composition of Squamate Reptiles: Relationship between Eggshell Permeability and Amino Acid Distribution. **Journal Of Chemical Ecology**, [s.l.], v. 31, n. 10, p.2391-2401, 28 set. 2005. Springer Nature. http://dx.doi.org/10.1007/s10886-005-7108-x.

TRACY, C. RICHARD et al. WATER RELATIONS OF CHELONIAN EGGS. **Physiological Zoology**, [s.l.], v. 51, n. 04, p.378-387, out. 1978.

TRACY, C. RICHARD. Water Relations of Parchment-shelled Lizard (Sceloporus undulatus) Eggs. **Copeia**, [s.l.], v. 1980, n. 03, p.478-482, set. 1980.

TRACY, C. R.; SNELL, H. L. Interrelations Among Water and Energy Relations of Reptilian Eggs, Embryos, and Hatchlings. **American Zoologist,** v. 25, n. 4, p. 999-1008, 1985.

YOUNG, J. D. The structure and some physical properties of the testudinian eggshell. **Proc. Zool. Soc.** London 120:455-469. 1950.

**References Chapter IV**

BAIN, M. M.; FAGAN, A.J.; MULLIN, J.M.; McNAUGHT, I.; McLEAN, J.; CONDON, B. Noninvasive Monitoring of Chick Development In Ovo Using a 7T MRI System From Day 12 of Incubation Through to Hatching. **Journal Of Magnetic Resonance Imaging**, [s.l.], v. 26, p.198-201, 2007.

BELINSKY, A. Water in Reptilian Eggs and Hatchlings *In:* Deeming, D. C. (ed.), **Reptilian Incubation. Environment, Evolution and Behaviour**. Nottingham, U.K: Nottingham University Press, 2004.

BLOCH, F. Nuclear Induction. **Physical Review**, [s.l.], v. 70, n. 07, p.460-474, 19 jul. 1946.

BLOCH, F.; GRAVES, A. C.; PACKARD, M.; SPENCE, R. W. Spin and Magnetic Moment of Tritium. **Physical Review**, [s.l.], v. 71, n. 06, p.373-374, 17 fev. 1947.

BLOEMBERGEN, N.; PURCELL, E. M.; POUND, R. V. Relaxation Effects in Nuclear Magnetic Resonance Absorytion. **Physical Review**, [s.l.], v. 73, n. 07, p.679-712, 01 abr. 1948.

BREED, F. S. The development of certain instincts and habits in chicks. **Pub. at Cambridge**, Boston, Mass., 1912.

BRISCOE, S. D.; ALBERTIN, C.B.; ROWELL, J. J. and RAGSDALE, C. W. Neocortical Association Cell Types in the Forebrain of Birds and Alligators. **Current Biology**, [s.l.], v. 28, n. 5, p.686-696, mar. 2018. Elsevier BV. http://dx.doi.org/10.1016/j.cub.2018.01.036.

BURTON, F. G.; TULLETT, S. G.. RESPIRATION OF AVIAN EMBRYOS. **Comp. Biochem. Physiol**, [s.l.], v. 82, n. 4, p.735-744, 1985.

CHANG, Y.; CHEN, P. Hierarchical structure and mechanical properties of snake (*Naja atra*) and turtle (*Ocadia sinensis*) eggshells. **Acta Biomaterialia**, [s.l.], v. 31, p.33-49, fev. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.actbio.2015.11.040.

COLLIAS, N. E. The Development of Social Behavior in Birds. **The Auk**, [s.l.], v. 69, n. 2, p.127-159, abr. 1952. JSTOR. http://dx.doi.org/10.2307/4081265.

DALE, B. M.; BROWN, M. A.; SEMELKA, R. C. **MRI: basic principles and applications**. John Wiley & Sons, 2015.

DAMADIAN, R. Tumor Detection by Nuclear Magnetic Resonance. **Science**, [s.l.], v. 171, p.1151-1153, 19 mar. 1971.

De IULIIS G, PULERA D (2007) **The dissection of vertebrates A laboratory manual**. London: Elsevier Academic Press.

DUNCKER, H. R. Development of the Avian Respiratory and Circulatory Systems. **Respiratory Function In Birds, Adult And Embryonic**, Berlin, p.260-273, 1978.

FALEN, S.W.; SZEVERENYI, N. M.; PAKARD Jr., D. S.; RUOCCO, M. J. Magnetic Resonance Imaging Study of the Structure of the Yolk in the Developing Avian Egg. **Journal Of Morphology**, [s.l.], v. 209, p.331-342, 1991.

FEDER M.E.; SATEL S.L.; GIBBS A.G. Resistance of the Shell membrane and mineral layer to diffusion of oxigen and water inflexibles shelled eggs of the snapping turtle (*Chelydra serpentina*). **Respir. Physiol**. 49:279-291. 1982.

FILOGONIO, R. et al. "Distribution of populations of broad-snouted caiman (Caiman latirostris, Daudin 1802, Alligatoridae) in the São Francisco River basin, Brazil. **Braz. J. Biol.**, [s.l.], v. 70, n. 4, p.961-968, nov. 2010.

HANSEN, Kasper et al. Magnetic Resonance Imaging Volumetry for Noninvasive Measures of Phenotypic Flexibility during Digestion in Burmese Pythons. **Physiological And Biochemical Zoology**, [s.l.], v. 86, n. 1, p.149-158, jan. 2013. University of Chicago Press. http://dx.doi.org/10.1086/668915.

HOGERS, B.; WEERD, L. van der.; OLOFSEN, H.; GRAAF, L. M. van der.; DeRUITER, M. C..; GITTENBERGER-DE GROOT, A.C.; POELMANN, R. E. Non-invasive tracking of avian development in vivo by MRI. **Nmr In Biomedicine**, [s.l.], v. 22, n. 4, p.365-373, maio 2009. Wiley-Blackwell. http://dx.doi.org/10.1002/nbm.1346.

KAUFFMAN, G. Nobel Prize for MRI Imaging Denied to Raymond V. Damadian a Decade Ago. **Chem. Educator**, [s.l.], v. 19, p.73-90, 21 mar. 2014.

KENT GC, CARR RK (2001) **Comparative anatomy of the vertebrates**, 9th edn. Singapore: McGraw-Hill.

KIRKMAN, F. B. The birth of a black-headed gull. **Brit. Birds**, 24: 283-291. 1931

KNOTEK, Z.; SIMPSON, S.; MARTELLI, P. Diagnostic Imaging. In: DONELEY, Bob et al. **Reptile Medicine and Surgery in Clinical Practice.** [s.l]: John Wiley & Sons Ltd, 2018. Cap. 12. p. 145-158.

KRYSKO, K. L. et al. Distribution, Natural History, and Impacts of the Introduced Green Iguana (*Iguana iguana*) in Florida. **Iguana Conservation**, [s.l.], v. 14, n. 3, p.142-151, set. 2007.

KUONI, W.; AUGUSTINY, N.; RÜBEL, A. Application of magnetic resonance imaging in reptile medicine. **Magma**, [s.l.], v. 1993, n. 1, p.61-63, 01 mar. 1993.

LOCY, W. A.; LARSELL, O. THE EMBRYOLOGY OF THE BIRD'S LUNG: Part I. **The American Journal Of Anatomy**, [s.l.], v. 19, n. 3, p.447-504, 1916a.

LOCY, W. A.; LARSELL, O. THE EMBRYOLOGY OF THE BIRD'S LUNG: Part II. **The American Journal Of Anatomy**, [s.l.], v. 20, n. 1, p.01-44, 1916b.

LOEBMANN, D.; HADDAD, C. F. B. Amphibians and reptiles from a highly diverse area of the Caatinga domain: composition and conservation implications. **Biota Neotropica**, [s.l.], v. 10, n. 3, p.227-256, set. 2010. FapUNIFESP (SciELO). http://dx.doi.org/10.1590/s1676-06032010000300026.

LOHMAN, J. A. B.; RATCLIFFE, R. G.. Prospects for NMR imaging in the study of biological morphogenesis. **Experientia**, [s.l.], v. 44, n. 1988, p.666-672, 1988.

MCCOSHEN, John A.; THOMPSON, Robert P.. A study of the effect of egg separation on hatching time and of the source of clicking sounds in the embryo of the domestic chicken. **Canadian Journal Of Zoology**, [s.l.], v. 46, n. 2, p.243-248, mar. 1968. Canadian Science Publishing. http://dx.doi.org/10.1139/z68-037.

MORRIS, K. A.; PACKARD, G.C.; BOARDMAN, T.J.; PAUKSTIS, G.L.; PACKARD, M.J. Effect of the Hydric Environment on Growth of Embryonic Snapping Turtles (*Chelydra serpentina*). **Herpetologica**, [s.l.], v. 39, n. 03, p.272-285, set. 1983.

NUNES, R.B. EVOLUÇÃO DA VIVIPARIDADE EM SQUAMATA: CENÁRIOS EVOLUTIVOS E RARIDADE DOS ESTÁGIOS INTERMEDIÁRIOS. **Revista da Biologia**, [s.l.], v. 01, n. 0, p.21-26, 04 nov. 2008.

PACKARD, G.C.; TAIGEN, T.L.; BOARDMAN, T.J.; PACKARD, M.J.; TRACY, C. R. Changes in Mass of Softshell Turtle (*Trionyx spiniferus*) Eggs Incubated on Substrates Differing in Water Potential. **Herpetologica,** [s. L.], v. 35, n. 01, p.78-86, mar. 1979.

PACKARD, G.C.; PACKARD, M.J.; BOARDMAN, T.J. PATTERNS AND POSSIBLE SIGNIFICANCE OF WATER EXCHANGE BY FLEXIBLE-SHELLED EGGS OF PAINTED TURTLES (*CHRYSEMYS PICTA*). **Physiological Zoology**, [s.l.], v. 54, n. 01, p.165-178, jan. 1981a.

PACKARD, G.C.; PACKARD, M.J.; BOARDMAN, T.J.; ASHEN, M. D. Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles. **Science.** [s.l.], p. 471-473. 22 abr. 1981b.

PACKARD, M.J.; PACKARD, G.C.; BOARDMAN, T.J. STRUCTURE OF EGGSHELLS AND WATER RELATIONS OF REPTILIAN EGGS. **Herpetologica,** [s. L.], v. 38, n. 1, p.136-155, mar. 1982.

PACKARD, G.C. Physiological and ecological importance of water to embryos of oviparous reptiles. In: Deeming, D.C., Ferguson, M.J.W. (Eds.), **Egg Incubation: Its Effect on Embryonic Development in Birds and Reptiles**. Cambridge University Press, New York pp. 213-228. 1991

PEEBLES, E. et al. Embryo and yolk compositional relationships in broiler hatching eggs during incubation. **Poultry Science**, [s.l.], v. 78, n. 10, p.1435-1442, 1 out. 1999. Oxford University Press (OUP). http://dx.doi.org/10.1093/ps/78.10.1435.

POUGH FH, HEISER JB, McFARLAND W (1996) **Vertebrate Life**. 4th edition. New Jersey: Prentice Hall.

RABI, I. I. Syace Quantization in a Gyrating Magnetic Field. **Physical Review**, [s.l.], v. 51, p.652-654, 15 abr. 1937.

RAITI, P.; HARAMATI, N. Magnetic Resonance Imaging and Computerized Tomography of a Gravid Leopard Tortoise (*Geochelone pardalis pardalis*) with Metabolic Bone Disease. **Journal Of Zoo And Wildlife Medicine**, [s.l.], v. 28, n. 02, p.189-197, jun. 1997.

ROCHA, C. F. D.; ANJOS, L.A.; BERGALLO, H.G. Conquering Brazil: the invasion by the exotic gekkonid lizard *Hemidactylus mabouia* (Squamata) in Brazilian natural environments. **Zoologia**, [s.l.], v. 28, n. 06, p.747-754, dez. 2011.

ROCHA, C.F.D; BERGALLO, H.G. Occurrence and distribution of the exotic lizard *Hemidactylus mabouia* Moreau de Jonnès, 1818 in Ilha Grande, RJ, Brazil. **Brazilian Journal Of Biology**, [s.l.], v. 71, n. 2, p.447-450, maio 2011. FapUNIFESP (SciELO). http://dx.doi.org/10.1590/s1519-69842011000300014.

RODRÍGUEZ, A.O.. Principles of magnetic resonance imaging. **Revista Mexicana de FÍsica**, [s.l.], v. 50, n. 03, p.272-286, jun. 2004.

SARTORI, M.R.; TAYLOR, E.W.; ABE, A.S.. Nitrogen excretion during embryonic development of the green iguana, *Iguana iguana* (Reptilia; Squamata). **Comparative Biochemistry And Physiology, Part A.** [s. L.], p. 210-214. 08 jun. 2012.

SIFUENTES-ROMERO, I.; TEZAK, B.M.; MILTON, S.L.; WYNEKEN, J. Hydric environmental effects on turtle development and sex ratio. **Zoology**, [s.l.], v. 126, p.89-97, fev. 2018. Elsevier BV. http://dx.doi.org/10.1016/j.zool.2017.11.009.

SUMMA, N.M.; RISI, E.E.; FUSELLIER, M.; SANCHEZ-MIGALLON, D. G.; ZWINGENBERGER, A.L.; MADEC, S.; RAPHAËL, C.; BETTI, E.R.P. Magnetic Resonance Imaging and Cross-Sectional Anatomy of the Coelomic Cavity in a Red-Eared Slider (*Trachemys scripta elegans*) and Yellow-Bellied Sliders (*Trachemys scripta scripta*). **Journal Of Herpetological Medicine And Surgery**, [s.l.], v. 22, n. 3-4, p.107-116, set. 2012. Association of Reptilian and Amphibian Veterinarians (ARAV). http://dx.doi.org/10.5818/1529-9651-22.3.107.

STRAUB, J.; JURINA, K. Magnetic Resonance Imaging in Chelonians. **Seminars In Avian And Exotic Pet Medicine**, [s.l.], v. 10, n. 4, p.181-186, out. 2001.

TRACY, C.R.; PACKARD, G.C.; PACKARD, M.J. WATER RELATIONS OF CHELONIAN EGGS. **Physiological Zoology**, [s.l.], v. 51, n. 04, p.378-387, out. 1978.

TRACY, C.R. Water Relations of Parchment-Shelled Lizard (*Sceloporus undulatus*) Eggs. **Copeia**, [s.l.], v. 1980, n. 03, p.478-482, set. 1980.

TRACY, C. R.; SNELL, H. L. Interrelations Among Water and Energy Relations of Reptilian Eggs, Embryos, and Hatchlings. **American Zoologist,** v. 25, n. 4, p. 999-1008, 1985.

VERDADE, L. M. Biologia Reprodutiva do Jacaré-de-Papo-Amarelo (*Caiman latirostris*) em São Paulo, Brasil. **Conservación y Manejo de Los Crocodylia de America Latina**, [s.l.], v. 1, p.57-79, 1995.

VERGNE, A. L.; MATHEVON, N. Crocodile egg sounds signal hatching time. **Current Biology**, [s.l.], v. 18, n. 12, p.13-14, jun. 2008. Elsevier BV. http://dx.doi.org/10.1016/j.cub.2008.04.011.

VERGNE, A. L.; PRITZ, M. B.; MATHEVON, N.. Acoustic communication in crocodilians: from behaviour to brain. **Biological Reviews**, [s.l.], v. 84, n. 3, p.391-411, ago. 2009. Wiley. http://dx.doi.org/10.1111/j.1469-185x.2009.00079.x.

VERGNE, A. L.; AUBIN, T.; TAYLOR, P.; MATHEVON, N. Acoustic signals of baby black caimans. **Zoology**, [s.l.], v. 114, n. 6, p.313-320, dez. 2011. Elsevier BV. http://dx.doi.org/10.1016/j.zool.2011.07.003.

VINCE, M. A. Synchronization of Hatching in American Bobwhite Quail (*Colinus virginianus*). **Nature**, [s.l.], v. 203, n. 4950, p.1192-1193, set. 1964a. Springer Nature. http://dx.doi.org/10.1038/2031192a0.

VINCE, M.A. Social facilitation of hatching in the bobwhite quail. **Animal Behaviour**, [s.l.], v. 12, n. 4, p.531-534, out. 1964b. Elsevier BV. http://dx.doi.org/10.1016/0003-3472(64)90075-2.

VINCE, M. A. Potential stimulation produced by avian embryos. **Animal Behaviour**, [s.l.], v. 14, n. 1, p.34-40, jan. 1966. Elsevier BV. http://dx.doi.org/10.1016/s0003-3472(66)80007-6.

WEAVER, Richard Lee. Reproduction in English Sparrows. **The Auk**, [s.l.], v. 60, n. 1, p.62-74, jan. 1943. JSTOR. http://dx.doi.org/10.2307/4079314.

WOODALL P.F. The structure and some functional aspects of the eggshell of broad-shelled river tortoise *Chelodina expansa* (Testudinata: Chelidae). **Aust. J. Zool.** 32:7-14. 1984.