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CAMILO ANDRES GONZALEZ GONZALEZ

ASPECTOS REPRODUTIVOS DE UMA POPULAÇÃO DE GUARÁ (*Eudocimus ruber*) SOB CUIDADOS HUMANOS EM BAIXA LATITUDE

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Tese apresentado ao Programa de Pós-Graduação em Reprodução Animal na Amazônia da Universidade Federal Rural da Amazônia para a obtenção do título de Doutorado.

Área de Concentração
Reprodução de Animais Silvestres e Domésticos.

Orientador
Prof.ª Dr.ª Sheyla Farhayldes Souza Domingues

Co-orientador
Prof. Dr. Ricardo José Garcia Pereira

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Belém, 25 de julho de 2024
CEUA N 7584300323
(ID 001712)

Ilmo(a). Sr(a).
Responsável: Sheyla Farhayldes Souza Domingues
Área: Medicina Veterinária

Título da proposta: "Caracterização da microbiota fecal de reprodutoras, da microbiota do ovo e da microbiota do embrião associadas a índices reprodutivos numa população em cativeiro de guará (Eudocimus ruber) no período de postura".

CERTIFICADO (Emenda versão de 18/junho/2024)

A Comissão de Ética no Uso de Animais da Universidade Federal do Pará, no cumprimento das suas atribuições, analisou e **APROVOU** a Emenda (versão de 18/junho/2024) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "Será feita uma nova metodologia sem alterar o N amostral nem as condições de manutenção das aves estudadas. A mudança será que os ovos ovipostos pelos animais de estudo serão abertos descrevendo cada estágio do desenvolvimento embrionário para espécie até antes da eclosão. Os embriões serão eutanasiados conforme orientação técnica do CFMV (vide documento anexo). Nenhum animal será eutanasiado, só embriões em desenvolvimento. Este estudo é de grande importância para melhorar o diagnóstico de problemas na incubação para espécie o que irá ser uma referência para qualquer instituição buscando aumentar a eficiência reprodutiva para espécie. ".

Nova previsão de término da proposta: **03/2024**

Comentário da CEUA: Será realizada alteração na metodologia da pesquisa, sem alterar o N amostral nem as condições de manutenção das aves. Considero que a proposta está ok e pode ser aprovada.

Profa. Dra. Barbarella de Matos Macchi
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal do Pará

Prof. Dr. James Tony Lee
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal do Pará

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À banca avaliadora por aceitar gentilmente o convite.
À Capes pela bolsa de estudos concedida.

SUMÁRIO

RESUMO DA TESE

GONZALEZ, C. A. G. **Aspectos reprodutivos de uma população de guará (*Eudocimus ruber*) sob cuidados humanos em baixa latitude.** 2025. 82f. Tese (Doutorado em Reprodução Animal na Amazônia). Instituto de Saúde e Produção Animal, Universidade Federal Rural da Amazônia, 2025.

A presente tese foi dividida em dois capítulos. No primeiro capítulo, objetivou-se descrever o desenvolvimento embrionário do guará vermelho semi-altricial (Eudocimus ruber), identificando marcos críticos para fins de diagnóstico e determinação de idade, apoiando tanto a incubação artificial quanto a pesquisa de campo da espécie. Utilizamos ovos coletados de uma população cativa no norte do Brasil. Um total de 50 embriões foram analisados do dia 0 ao dia 18, com foco nas características morfológicas externas observáveis através do exame macroscópico. As principais características diagnósticas incluíram a formação sequencial do bico, olhos, pescoço, penas e anexos embrionários. Comparamos o desenvolvimento do guará vermelho semi-altricial com descrições de referência de espécies precoces e altriciais para avaliar a adequação das comparações entre diferentes padrões de crescimento pós-natal. O guará vermelho semi-altricial exibiu variações heterocrônicas de até quatro dias em marcos críticos de desenvolvimento em comparação com espécies precoces com períodos de incubação semelhantes. Estas descobertas sugerem que os cronogramas de desenvolvimento estabelecidos para espécies precoces podem não ser confiáveis para o estadiamento preciso de embriões altriciais e semi-altriciais. O tempo médio de eventos de desenvolvimento significativos no guará vermelho alinhou-se mais estreitamente com os de outras espécies altriciais. Os marcos embrionários diários aqui descritos fornecem uma referência valiosa para programas de reprodução em cativeiro de guará e contribuem para esforços de conservação mais amplos para outros membros da família *Threskiornithidae* ou espécies dentro da ordem Pelecaniformes, abordando a pesquisa limitada sobre o desenvolvimento de aves semi-altriciais. No segundo capítulo, objetivou-se descrever o desempenho reprodutivo de uma população cativa de guarás ao longo de um período de três anos (2022, 2023 e 2024). Os fatores avaliados incluem o início e a conclusão da estação reprodutiva, formação de casais, densidade de recintos e tempo de postura. Conduzimos análises estatísticas para avaliar o impacto de fatores relacionados aos reprodutores (como idade e período de postura), características dos ovos (incluindo peso e formato) e parâmetros de incubação (como perda de peso dos ovos durante a incubação), em variáveis zootécnicas importantes comumente usadas na produção avícola, incluindo taxa de postura, fertilidade e eclodibilidade. Visando estabelecer um perfil de reprodutor que pudesse orientar a seleção de matrizes para programas reprodutivos na espécie. O início da estação reprodutiva variou entre julho e agosto ao longo dos três anos, enquanto sua conclusão variou de novembro a janeiro. Tanto os machos quanto as fêmeas começaram a formar pares reprodutivos já aos dois anos de idade, com as idades mais velhas de pareamento sendo 20 anos para os machos e 12 anos para as fêmeas. 82,6 ± 4.9% dos pares foram monogâmicos durante toda a temporada. Maiores densidades de indivíduos no recinto parecem melhorar as taxas de postura e encorajar posturas mais precoces, enquanto uma proporção de 1:1 entre machos e fêmeas facilita a formação efetiva de pares com agressão mínima. Além disso, o aumento da idade das fêmeas está correlacionado com uma maior taxa de postura ($p < 0,05$). A retirada dos ovos estimulou uma nova postura de ovos nas fêmeas, resultando em uma média de $7,6 \pm 2,7$ novos ovos. O avanço da estação de postura influenciou significativamente o peso inicial dos ovos ($p < 0,01$). A perda média de peso diária durante a incubação foi de 0,74% (tau = 0,5), enquanto a perda média de peso no dia 21 foi de 19 ± 2%. As descobertas deste estudo são inestimáveis para o planejamento de programas de reprodução eficientes para o guará e potencialmente para outros membros da família Threskiornithidae.

Palavras-chave: Aves, reprodução, incubação, íbis, conservação.

ABSTRACT OF THESIS

GONZALEZ, C. A. G. **Reproductive aspects of a population of maned (Eudocimus ruber) under human care at low latitude.** 2025. 82f. Thesis (Doctorate in Animal Reproduction in the Amazon). Institute of Animal Health and Production, Federal Rural University of the Amazon, 2025.

This thesis was divided into two chapters. In the first chapter, the objective was to describe the embryonic development of semi-altricial scarlet ibis (*Eudocimus ruber*) while identifying critical landmarks for diagnostic and age determination purposes, supporting both artificial incubation and field research on the species. We used eggs collected from a captive population in northern Brazil. A total of 50 embryos were analyzed from day 0 to day 18, focusing on external morphological traits observable through macroscopic examination. Key diagnostic features included the sequential formation of the beak, eyes, neck, feathers, and embryonic annexes. We compared the development of the semi-altricial scarlet ibis with reference descriptions of precocial and altricial species to evaluate the suitability of comparisons between different postnatal growth patterns. The semi-altricial scarlet ibis exhibited heterochronic variations of up to four days in critical developmental landmarks compared to precocial species with similar incubation periods. These findings suggest that developmental timelines established for precocial species may not be reliable for accurately staging altricial and semi-altricial embryos. Interestingly, the median timing of significant developmental events in the scarlet ibis aligned more closely with those of other altricial species. The daily embryonic milestones described herein provide a valuable reference for scarlet ibis captive breeding programs and contribute to broader conservation efforts for other Threskiornithidae family members or species within the Pelecaniformes order, addressing the limited research on semi-altricial avian development. In the second chapter, we aimed to describe the breeding performance of a captive population of scarlet ibis over a three-year period (2022, 2023, and 2024). We focused on various factors, including the onset and conclusion of the breeding season, couple formation, enclosure density, and laying timing. We conducted statistical analyses to assess the impact of breeder-related factors (such as age and laying timing), egg characteristics (including weight and shape), and incubation parameters (such as egg weight loss) on key zootechnical variables commonly used in poultry production, including laying rate, fertility, and hatchability. Our goal was to establish a breeder profile that could inform mate selection. The onset of the breeding season varied between July and August over the three years, while its conclusion ranged from November to January. Both males and females began forming breeding pairs as early as two years old, with the oldest pairing ages being 20 years for males and 12 years for females. 82,6 ± 4.9% of pairs were monogamous throughout the season. Higher enclosure densities appeared to improve laying rates and encourage earlier laying, while a 1:1 male-to-female ratio facilitated effective pair formation with minimal aggression. Additionally, increased female age correlated with a higher laying rate ($p < 0.05$). Egg withdrawal stimulated the replacement of eggs in females, resulting in an average of 7.6 ± 2.7 new eggs. The advancement of the laying season significantly influenced initial egg weight ($p < 0.01$). The median daily weight loss during incubation was 0.74% (tau = 0.5), while the mean weight loss on day 21 was 19 ± 2%. The findings from this study are invaluable for planning efficient breeding programs for the scarlet ibis and potentially for other members of the

Threskiornithidae family.
Key words: Birds, breeding, incubation, ibis, conservation.

1 CONTEXTUALIZAÇÃO

1.1 O GUARÁ (*Eudocimus ruber*)

O guará pertence junto com os outros íbis à família Threskiornithidae que inclui 28 espécies. O estado de conservação varia entre as espécies estando algumas com população estável e outras com algum grau de ameaça até criticamente ameaçados segundo a IUCN (International Union for Conservation of Nature). A experiência obtida com a criação de espécies mantidas em zoológicos pode ser utilizada para aprimorar os esforços de conservação de espécies em perigo de extinção. Embora se tenha menos preocupação de acordo com a IUCN, a população de guará está atualmente em declínio (Birdlife International, 2012; Miranda et al., 2021), e a espécie é considerada extinta em diversas regiões do Brasil (Sick, 1997; Ebird, 2015).

1.2 BIOLOGIA E COMPORTAMENTO REPRODUTIVO

Os guarás são animais de pernas e pescoço longos e ressaltam na natureza por apresentarem uma exuberante plumagem vermelha decorrente da dieta rica em carotenóide cantaxantina, presente na casca do caranguejo maracoani ou chama-maré, sua principal fonte de alimento (**Figura 1**). As pontas de suas pontas mais longas e seus olhos são de cor preta. O bico destes animais tem um gradiente de cor de acordo com a idade e sexo (Sick, 1997). Suas pernas possuem escamas e os seus dedos ligam-se ligeiramente uns com os outros por uma membrana interdigital, permitindo que os mesmos possam pousar em árvores (Scherer e Baldin, 2014).

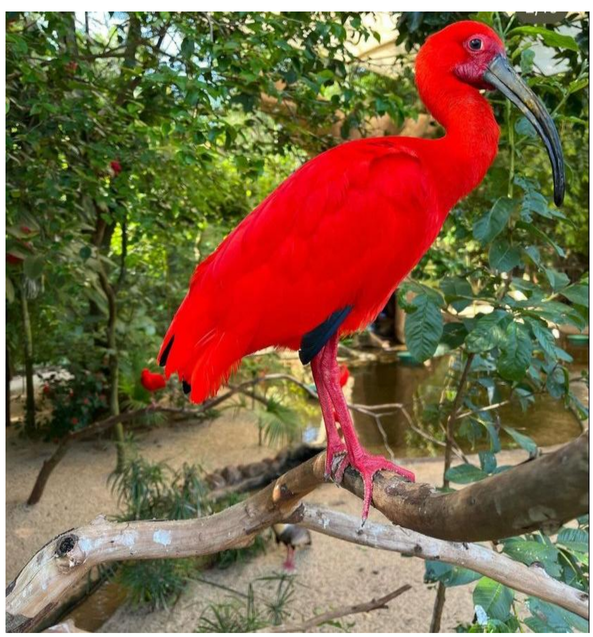

Figura 1Indivíduo adulto de guará (*Eudocimus ruber*).

Esta espécie pode ser encontrada em manguezais, pântanos e diversas áreas úmidas da América do Sul. Entretanto a população destes animais vem diminuindo gradativamente apesar de seu estado de conservação ser pouco alarmante (Birdlife international, 2012). Entre os principais fatores que afetam essas populações estão entre eles, a perda de habitat, a destruição das áreas de nidificação e de alimentação, a caça excessiva, o comércio ilegal, colheita dos ovos e venda das penas para fins de confecção de adornos (IPA, 2009).

Os guarás alcançam a maturidade sexual com 2 anos e tem registros da primeira postura com 2.5 anos. A máxima longevidade registrada é de uma fêmea no zoo de Amsterdam com 33 anos e 3 meses (García et al., 2014). A postura do guará varia entre 1 a 3 ovos verde escuro com manchas marrom. Os ovos são incubados e os pintinhos protegidos e alimentados por ambos país de forma alternada. O período de incubação vai de 21 a 23 dias (Ramo e Busto, 1985; Miranda et al., 2021). Os filhotes são semi-altriciais, apresentando pouco movimento nos primeiros 10 dias e comportamentos exploratórios a partir do Dia 12. Abandonam o ninho entre 35 e 42 dias após a eclosão (Miranda et al., 2021).

1.3 ESTUDO DE REPRODUÇÃO EM GUARÁ – O ESTADO DA ARTE

O primeiro reporte de reprodução em cativeiro do guará foi no Zoo de Berlim em 1881 (Brouwer et al 1994). Continua sendo reproduzido com sucesso em diversos zoos ao redor do mundo contanto atualmente com um Stud Book de Norte América para espécie. No zoológico de Tóquio tem sido feitos diversos estudos na reprodução em cativeiro do guará e de outros íbis o que tem auxiliado na conservação do Guará de Crista Japonês (García et al., 2014). Samayah em 2009 reportou uma postura média de 1.98 ± 1.8 no pantanal de Caroni, Trinidad e Tobago. Do total 171 ovos, 127 ou 74% eclodiram. Em cativeiro, o tamanho do grupo é um fator determinante do sucesso reprodutivo de guarás. Colônias de menos de 32 indivíduos tem menos filhotes em méDia ou não produzem filhotes segundo Elbin e Lyles (1994). Estes autores recomendam uma proporção macho: fêmea de 1:1.6.

No Vogelpark Walsrode (Alemanha) foi desenvolvido um protocolo de coleta de ovos de guará quando a maioria dos casais já estão incubando. Os ovos são coletados em 2 ou 3 intervalos o que estimula novas ninhadas nos reprodutores. Dois casais de guará produziram 20 juvenis em 3 anos (média de 6.6 pintinhos por ano). Em 5 anos, seis pares produziram 106 juvenis sendo o registro de maior quantidade de posturas para um casal da espécie (García et al., 2014).

Em relação a incubação artificial tem registros de incubação de ovo de guará em temperaturas de 37,2°C a 37,5ºC e umidades de 50% a 65% (Ryder and Manry 1994, García et al., 2014, Miranda et al., 2021).

No Parque Mangal das Garças foram desenvolvidas outras pesquisas com a espécie como a realizada por Miranda et al. (2015), que avaliaram o desenvolvimento de filhotes submetidos a diferentes dietas, além de desenvolver um protocolo para criação destes animais em cativeiro avaliando periodicamente seu crescimento. Além deste, Silva (2015), realizou pesquisa sobre a caracterização de bactérias e leveduras em fezes de guará, evidenciando a importância de se compreender a epidemiologia de doenças comuns em aves silvestres e que podem acometer seres humanos. Sendo assim, o presente trabalho traz informações ao respeito da fisiologia reprodutiva destes animais, que ainda não foram abordados anteriormente por nenhum estudo no país, que podendo assim contribuir consideravelmente para o desenvolvimento programas reprodutivos da espécie em cativeiro.

1.4 BIOTÉCNICAS DE REPRODUÇÃO NA AVICULTURA INDUSTRIAL

A avicultura industrial tem tido um grande avanço técnico para otimizar a eficiência do sistema produtivo. Embora o processo de desenvolvimento e eclosão de embriões aviários seja bastante similar entre espécies, ainda existe uma diferencia considerável da produção avícola industrial para outros sistemas de produção e mais ainda para a reprodução de espécies silvestres em cativeiro (Pereira, Blank e Barbosa, 2021). Variações importantes entre espécies são vistas em períodos de incubação, com exigências de umidade e temperatura e no processo de eclosão (Kasielke, 2020).

Pereira, Blank e Barbosa (2021), sugerem que uma abordagem zootécnica na reprodução de aves silvestres orientada à otimização da produção de pintinhos viáveis é fundamental não só para incrementar a produção de espécies raras e ameaçadas, mas também para criar a infraestrutura necessária para tornar esta atividade cada vez mais rentável.

Os dois indicadores de eficiência reprodutiva mais utilizados nesta abordagem zootécnica são a fertilidade e a eclodibilidade que são expressos como porcentagens. A fertilidade é a proporção de ovos férteis do total de ovos ovipostos. Na avicultura industrial este indicador usualmente é de 90% ou superior. A eclodibilidade é a proporção de ovos eclodidos do total de ovos férteis que foram incubados. Na avicultura industrial está entre 87- 93% geralmente (King'ori, 2011; Taplah et al., 2018). Tanto a fertilidade quanto a eclodibilidade em espécies domésticas podem ser afetadas por fatores genéticos e não genéticos. Dentre os fatores encontram-se o manejo do plantel reprodutivo, seleção genética, idade dos reprodutores, nutrição e qualidade do ovo (Drouilhet et al., 2014; Hester, 2017).

A fertilidade em específico, pode ser afetada pela qualidade do plantel reprodutivo, a proporção de macho: fêmea, a temperatura ambiental, o tempo de armazenamento do ovo e os recintos (Chowdhury et al., 2004). A idade da fêmea tem mais influência na fertilidade que a idade dos machos (Brommer and Rattiste, 2008). Além disso, a idade das matrizes afeta a qualidade interna e externa dos ovos, influenciando fertilidade e eclodibilidade (Mourão et al., 2010).

Por outro lado, a eclodibilidade é afetada por fatores como genética, nutrição, exposição a toxinas, doenças e idade dos pais, estresse ou trauma físico que afeta a formação do ovo. (Landauer 1967; Romanoff and Romanoff 1972; Kuehler 1983). Adicionalmente, fatores ambientais relacionados com a incubação como a temperatura, umidade relativa, ventilação e viragem dos ovos também influenciam a eclodibilidade em diversas espécies (Archer et al., 2017; Ramli et al., 2017)

A idade da fêmea afeta a deposição de cálcio e minerais na casca (Onbaşılar et al., 2014). A casca permite a troca gasosa e a perda de umidade do ovo, portanto, uma casca muito grossa pode interferir na troca gasosa e na perda de calor e uma casca muito fina pode facilitar a perda excessiva de umidade relativa durante a incubação (Peebles et al., 2001; Jibrin et al., 2011). Estudos em diversas espécies correlacionam a idade da fêmea com o peso do ovo e a eclodibilidade, sendo que fêmeas muito novas ou muito velhas apresentam uma menor eclodibilidade dos seus ovos (Cabezas- Díaz et al., 2005).

Em aves domésticas e algumas outras espécies, o peso e o índice de forma do ovo pode ter uma influência significativa na eclodibilidade (Whiting e Pesti, 1983; Senapati et al., 1996; Narushin e Romanov, 2002). Um aumento na espessura da casca e no conteúdo interno do ovo leva a um peso total maior, o que reflete uma maior reserva de nutrientes e energia (Toro et al., 2015). Embriões maiores produzem mais calor pelo que precisam uma boa ventilação e correta temperatura (Jibrin et al., 2011). Dependendo da espécie, ovos com formas mais alongadas ou redondas, ou com peso muito distante da média, tem menor probabilidade de eclodir (Narushin and Romanov, 2002). Weis et al. em 2011 encontraram dados confirmatórios de que ovos de tamanho médio de pato tinham melhor eclodibilidade que ovos pequenos ou muito grandes. Por outro lado, Mourão et al. (2010) não encontraram correlação entre a forma do ovo e a eclodibilidade em perdizes vermelhas.

Uma prática comum na avicultura industrial é a retirada frequente de ovos do ninho das reprodutoras. Com isto busca-se reduzir o estímulo visual e táctil da mãe com o ovo evitando a diminuição dos níveis de estrogênio e aumento dos níveis de prolactina que por consequência causariam uma inibição da postura e um estímulo do comportamento de choco. Retirando os ovos várias vezes por Dia é possível aumentar de forma considerável a quantidade de ovos postos por matriz (Riou, 2010; Angelier et al., 2013; Pereira, Blank e Barbosa, 2021). Este manejo amplamente usado na avicultura tem sido aplicado com sucesso na reprodução em cativeiro de algumas espécies ameaçadas, podendo obter um maior número de ovos e permitindo o desenvolvimento dos pintinhos sob condições mais controladas (Kuehler e Witman, 1988; Boyce et al., 2005, Keefer e Songsasen, 2019), no entanto alguns autores como Nager et al. (1999) e Salamon e Kent (2013) tem observado uma diminuição do tamanho do ovo como resultado de uma depleção de nutrientes conforme mais ovos são retirados da mesma matriz.

Outra prática rotineira na avicultura industrial é o armazenamento de ovos por alguns dias antes de entrar na incubadora, o que é um fator crítico de sucesso na produção, reduzindo custos, facilitando a logística de transporte e sincronizando os nascimentos de muitos pintinhos (Fasenko, 2007; Araújo et al., 2015; Pereira, Blank e Barbosa, 2021). Para evitar o efeito do estresse térmico no embrião durante o armazenamento a temperatura deve estar entre 13 e 18ºC, sendo que com temperaturas de 21ºC ou maior, o desenvolvimento embrionário inicia, porém, os tecidos podem se formar a diferentes tempos resultando em morte embrionária (Kasielke, 2020). Os ovos também irão perder muito peso por evaporação de água se mantidos a umidade ambiental pelo que a umidade deve ser mantida entre 40 e 75% (Buhr, 1995).

O tempo de armazenamento antes da incubação tem um efeito significativo na eclodibilidade e a mortalidade embrionária inicial (Waehner et al., 2015). Reyna e Burggren (2017) reportaram uma diminuição na fertilidade em ovos de pato armazenados por mais de 6 Dias após a postura. Para um melhor sucesso na eclosão os ovos são armazenados e incubados com o polo fino para baixo, provavelmente por uma melhor capacidade do embrião de eliminar água nesta orientação já que o embrião fica em contato com a câmera de ar (Kasielke, 2020). A ventilação não é tão determinante, mas é importante evitar a condensação de umidade sobre os ovos. Durante o armazenamento os ovos devem ser girados pelo menos uma vez por dia num ângulo de 90º. Os ovos armazenados devem ser aquecidos gradualmente antes de ser colocados na incubadora. (Kasielke, 2020).

Muitas famílias como aves aquáticas, não começam incubar os ovos até que a postura esteja completa. Ovos destas espécies toleram melhor curtos períodos de armazenamento que outras espécies que incubam só um ovo ou que iniciam a incubação com o primeiro ovo ovipostos. Estes ovos podem ser armazenados até por 7 dias sem diminuição da eclodibilidade (Kasielke, 2020).

1.5 DESENVOLVIMENTO DO EMBRIÃO: PERDA DE PESO E EMBRIODIAGNÓSTICO

O desenvolvimento dos filhotes de ave está dividido em quatro categorias: precocial (indivíduo com capacidade de locomoção e alimentação logo após a eclosão), altricial (indivíduos sem capacidade de locomoção e alimentação autônoma nas fases iniciais) e as categorias intermediárias semi-altricial e semiprecocial. A composição inicial do ovo está relacionada com esta classificação, sendo que em espécies precociais a gema possui um tamanho maior em comparação ao albume, o que confere uma maior disponibilidade de energia para o desenvolvimento prévio à eclosão e uma menor quantidade de água. Em espécies altriciais a proporção de água dentro do ovo é maior e a de energia menor (Tazawa e Whittow, 1999; Vaz Guida, 2018).

Após a postura, no ovo fértil o embrião ou blastoderma, encontra-se posicionado na região menos densa da gema pelo que irá ocupar o ponto mais alto independente da orientação do ovo durante o desenvolvimento inicial (Kasielke, 2020). A gema é cercada por camadas de albúmen, incluindo a calaza, que mantem a gema suspensa no centro do ovo e previne que o embrião se adira à casca. O albume serve como uma fonte de água e proteína para o embrião e contém imunoglobulinas que funcionam como imunidade passiva (Vaz Guida, 2018). O albume é cercado pela membrana interna e externa da casca. No polo largo do ovo entre estas duas membranas se forma a câmera de ar, isto devido a contração do conteúdo do ovo causada pela diferencia de temperatura interna da fêmea e do ambiente na hora da postura. A casca fornece proteção física e é uma fonte primaria de cálcio e outros minerais para o embrião em desenvolvimento. A casca é coberta por uma camada chamada de cutícula que auxilia regular a perda de água além de ser uma barreira mecânica contra patógenos (Kasielke, 2020).

O desenvolvimento embrionário pode ser dividido em duas fases: a fase de diferenciação onde são formadas a maioria de estruturas embrionárias e extraembrionárias, e a fase de crescimento, onde o embrião aumenta de tamanho e os tecidos se desenvolvem até a eclosão. Na galinha a primeira fase vai até o dia 12 de desenvolvimento e a segunda fase até o dia 21, dia aproximado da eclosão (Deeming, 2002; Vaz Guida, 2018).

Durante a incubação os ovos devem perder uma quantidade determinada de peso decorrente da evaporação de água através dos poros da casca. Para a maioria das espécies de aves a eclosão acontece com uma perda de 12 a 15% do peso inicial do ovo (Pereira, Blank e Barbosa, 2021). Por ser um processo físico de evaporação, a perda de peso do ovo pode ser regulada modificando as condições de temperatura e umidade na incubação, o que tem influência significativa no sucesso da eclosão. Os ovos devem ser pesados imediatamente após a postura sempre que possível. Caso seja utilizado como peso inicial o peso de um ovo que já iniciou a incubação, o cálculo de perda de peso pode ser afetado, podendo também afetar a eclosão desse ovo. Idealmente os ovos devem ser pesados todos os dias na mesma hora, em especial durante os primeiros dias de incubação, já que com passar do tempo se torna mais difícil mudar a curva de perda de peso de um ovo. Em caso de haver uma grande quantidade de ovos, a média de perda de peso pode ser inferida por uma amostra representativa do total de ovos incubados (Kasielke, 2020).

O posicionamento correto na hora da eclosão depende de uma perda adequada de peso durante a incubação bem como da posição correta do ovo e de uma ventilação adequada (excesso de CO2 pode causar embriões de cabeça para baixo) (Kasielke, 2020). Em caso de morte embrionária, o exame dos embriões que não eclodiram ou também chamado de embriodiagnóstico, é fundamental para o sucesso das próximas eclosões (Joyner e Abbott, 1991; Ernst et al., 2004; Almeida et al., 2015). Com este procedimento é possível Diagnosticar problemas de fertilidade bem como identificar e corrigir falhas de manejo no incubatório durante cada estágio de desenvolvimento do embrião (Thierry et al., 2013; Vaz Guida, 2018).

Existe uma mortalidade normal ou esperada em períodos específicos de desenvolvimento. Um terço da mortalidade esperada ocorre nos primeiros dias da incubação e se deve provavelmente a anomalias cromossômicas na formação dos gametas. Os dois terços restantes acontecem durante os últimos dias da incubação e são usualmente associados ao processo de eclosão, incluindo mal posicionamento. Deve existir pouca ou nenhuma mortalidade no meio desses dois períodos. (Kasielke, 2020)

Durante a necropsia do embrião é importante uma revisão da história dos pais e o registro de incubação. O ovo deve ser iluminado para localizar o embrião, a câmera de ar e outras características normais e anormais. Na abertura do ovo se pode confirmar a fertilidade do ovo observando na gema a presença de blastodisco, que é uma estrutura circular em forma de anel branco, presente nos ovos não fertilizados, entretanto que, no ovo fértil será possível observar a presença do embrião ou em estágios iniciais a presença de tecidos membranosos esbranquiçados sobre a superfície da gema. Diversos autores a partir de Hamburger e Hamilton (1951) seguem um padrão de classificação do embrião da galinha baseado no seu desenvolvimento onde a mortalidade do ovo fértil pode ser classificada como uma mortalidade embrionária inicial (≤ 3 Dias), mortalidade intermediaria (3-14 Dias) e mortalidade tardia (≥ 14 Dias). É importante determinar o estágio de desenvolvimento de cada embrião e confirmar que esteja concordante com o tempo de incubação do ovo. Em ovos com mortalidade tardia o posicionamento do embrião pode oferecer informação diagnostica relevante e deve ser registrado.

Em 1992 Hamburger e Hamilton formularam uma série de 46 estágios no desenvolvimento embrionário de galinhas que posteriormente serviu como base para estudar o desenvolvimento de algumas espécies domésticas e silvestres, tais como quiri-quiri (Pisenti et al., 2001), codorna japonesa (Ainsworth et al., 2010), pombo (Olea e Sandoval, 2012), pássaro mandarim (Murray et al., 2013) e ema (Almeida et al., 2015) entre outras.

CAPÍTULO I
DIFFERENT STAGES OF EMBRYONIC DEVELOPMENT IN SCARLET IBIS
(Eudocimus ruber)

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Different stages of embryonic development in scarlet ibis (*Eudocimus ruber*)

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Abstract

Efficient captive programs are crucial for the conservation of threatened avian species. Embryo diagnosis, a routine practice that enhances efficiency in industrial poultry farming, can be adapted to wild bird breeding management. This study aimed to describe the embryonic development of semi-altricial scarlet ibis (*Eudocimus ruber*) while identifying critical landmarks for diagnostic and age determination purposes, supporting both artificial incubation and field research on the species. We used eggs collected from a captive population in northern Brazil. A total of 50 embryos were analyzed from day 0 to day 18, focusing on external morphological traits observable through macroscopic examination. Key diagnostic features included the sequential formation of the beak, eyes, neck, feathers, and embryonic annexes. We compared the development of the semi-altricial scarlet ibis with reference descriptions of precocial and altricial species to evaluate the suitability of comparisons between different postnatal growth patterns. The semi-altricial scarlet ibis exhibited heterochronic variations of up to four days in critical developmental landmarks compared to precocial species with similar incubation periods. These findings suggest that developmental timelines established for precocial species may not be reliable for accurately staging altricial and semi-altricial embryos. Interestingly, the median timing of significant developmental events in the scarlet ibis aligned more closely with those of other altricial species. The daily embryonic milestones described herein provide a valuable reference for scarlet ibis captive breeding programs and contribute to broader conservation efforts for other Threskiornithidae family members or species within the Pelecaniformes order, addressing the limited research on semi-altricial avian development.

Key words: Birds, reproduction, captive breeding, artificial incubation, embryo diagnosis, semi-altricial development.

1 Introduction

The scarlet ibis and other ibis species belong to the order Pelecaniformes, Threskiornithidae family, which includes 13 genera and 36 species. Currently, 20% of these species are classified as endangered or critically endangered according to the International Union for Conservation of Nature (IUCN, 2024). While the conservation status of scarlet ibis is considered less concerning, its global population is declining and the species is considered extinct in several regions of Brazil where has been included in the National List of Endangered Species (Olmos, 2011; Ebird, 2015;

Miranda et al., 2021). A few studies on the successful captive breeding of the scarlet ibis, have contributed to the conservation of threatened species within its family, such as the japanese crested ibis (García et al., 2014).

One of the main challenges in the captive reproduction of wild bird species is increasing the percentage of successful hatchlings, given the limited knowledge available on reproductive biology of each species (Pereira et al., 2021). Hatching success is one of the most important indicators of system efficiency. In industrial poultry farming, hatchings rates vary between 90% and 100% of incubated eggs. This high success rate is achieved through continuous improvement of hatchery processes and infrastructure, which are based on opportune diagnosis and correction of failures during the incubation program (Kingori, 2011; Brown et al., 2019)

Among the routine practices in industrial poultry, embryo diagnosis plays a crucial role for enhancing system efficiency (Vaz Guida, 2018). Initially, it was based on the chicken embryo stages defined by Hamburger and Hamilton (1951), which served as a reference for normal embryo development. It allowed accurate embryo aging and the prompt identification of abnormalities. As a result, a better understanding of the varying environmental requirements at each stage of development emerged. This made it possible to more precisely identify the probable cause of death at any given embryonic age and to adjust conditions accordingly, helping to prevent further losses in the incubation batch (Browne, 2006; Pereira et al., 2021).

However, the avian development stages have predominantly focused on the domestic chicken and other domestic precocial birds (Ainsworth et al., 2010; Almeida et al., 2015; Morini et al., 2023). Studies examining post-natal growth patterns along the altricial-precocial spectrum suggest that the precocial charts do not fully capture the embryo developmental patterns of all bird species (Blom, 2005; Vaz Guida, 2018; Cordero and Werneburg, 2022). Furthermore, accurate aging and detailed assessment of normal and abnormal embryo development require references that are taxonomically close to the species being examined, ideally using species-specific standards in order to establish specific incubation programs (Murray, 2013).

The scarlet ibis belongs to the semi-altricial type II post-natal growth pattern characterized for having closed eyes when hatching (Rickleffs, 2000). Descriptions for this group are scarce (Herbert, 1967; Köppl, et al., 2005; Olea and Sandoval, 2012). Similarly, to date, there are no studies describing embryo development on other members from Threskiornithidae family, nor is there any description of another species from the Pelecaniformes order, apart from works on cormorant embryo development from Price (1938), Handbidge and Fox (1996), and Powell et al (1998).

Therefore, the aims of this work are a) to describe a timeline of daily embryo development of the scarlet ibis, with focus on external visible characters that can serve as a practical diagnostic tool for species-specific incubation programs. b) To compare some of the developmental stages of the scarlet ibis, as a semi-altricial species, to works describing precocial and altricial embryo development, enhancing the comprehension of aspects of each post-natal development pattern and highlight the potential for cross-species assessment of embryonic development.

2 Methods

2.1 Ethical aspects

All experimental procedures in this work plan were approved by the UFPA Animal Research Ethics Committee (CEUA Nº 7584300323).

2.2 Location and period of study

The study was conducted in the Mangal das Garças Zoo Park located in the city of Belém, state of Pará-Brazil, 1°27'48.9"S 48°30'17.8"W. AM-SEMAS Nº 544879. The study was conducted during the 2024 breeding season, which started in June and runs until the end of January. Recorded temperature range during this period was 24.4–33.6°C. As expected for a low-latitude location, daylight hours exhibited minimal variation across the year. The date of the first egg laid until the date of the last egg in the series was considered the total length of the breeding season.

2.3 Study animals

The study was conducted with eggs laid by a group of 36 scarlet ibis (18 males and 18 females) previously sexed by molecular tests, aged between 2 and 20 years old, born in captivity in the zoo's species breeding program. The enclosures have concrete and sand substrate, artificial lake, natural perches, and natural light in the morning and afternoon. Artificial nests were installed after observing courtship behavior, being made of wood and plastic mesh at the bottom in order to reduce the risk of broken eggs. A video recording from a single camera positioned inside each enclosure, partially captured the nesting and breeding behavior of the pairs. The animals were fed with species-specific feed (Flamingo/guará Fl32 Megazoo ®, São Paulo, Brazil) containing 32% protein, 5% crude fiber, and 7% ether extract, among other components, in the amount of 50g/kg/animal once per day with scheduled supplements with a multivitamin complex + powdered probiotics (Organew - Vetnil ®) and mealworms.

2.4 Egg management

The nests were inspected 7 times a day from 06:00 to 18:00, with the aim of removing the eggs as quickly as possible after laying and thus reducing the brood stimulus in the breeders. The eggs were removed from the nest and the couple were identified according to prior marking in the matrices. The eggs were taken to the incubatory, sanitized with 70% alcohol and screened for fail in the eggshell, only eggs with any cracks in the shell were discarded. Each egg was weighed using an electronic scale with a sensitivity of 0.1g.

2.5 Incubation

The egg was identified with a pencil and placed in a digital incubator, with automatic turning programmed every two hours (IP 130 PS, Premium Ambiental Ltda., Belo Horizonte, Minas Gerais, Brazil). The temperature and humidity were programmed at 37.5°C and 60% respectively as recorded by García et al., (2014) for the species.

We weighed the eggs on days 0, 6, 12 and 19, when it was separated for hatching. We performed ovoscopy on each of these days to monitor embryo development and infertile eggs or dead embryos were discarded following embryo necropsy. Eggs showing no membrane development during candling were opened to assess fertility.

2.6 Description of embryonic development

To euthanize eggs at less than 50% of the incubation period, they were exposed to temperatures below 4ºC for 4h, inducing death by hypothermia (Vaz Guida, 2018; AVMA, 2013; CFMV, 2013). For embryos at more than 50% of the incubation period, a cotton ball soaked in 100% inhaled isoflurane (1ml/ml) was placed into the air chamber of the egg following shell removal, resulting in death of the embryo within approximately 5 minutes (Vaz Guida, 2018; Wayne State University, 2022).

We selected, whenever possible, 3 eggs for each day of embryonic development from Day 1 to Day 18 to describe the morphological characteristics. The daily events recorded were compared to a reference descriptions of embryo development stages in precocial chicken with the same incubation period (Hamburger and Hamilton, 1951), as well as description of stages from 2 altricial species of 14 and 28 days of incubation period respectively, resulting in a median of 21 days (Murray, 2013; Pisenti, 2001). A photographic record of the embryo was taken after removing it from the egg and submerging in water as described by Kasielke (2020), to register relevant morphological aspects such as the appearance of blood vessels and other morphological variations according to the age of embryonic development.

3 Results

During the breeding season, a total of 75 eggs were laid by 17 pairs. 7 eggs were broken prior to evaluation of fertility. Among the remaining eggs, 57 eggs were fertile, resulting in a fertility rate of 83,9%. In terms of embryo development, there were 9 early embryo deaths, 3 late embryo deaths and 6 instances of embryo abnormalities. A total of 50 embryos were evaluated from days 0 to 18.

The early morning visits to the nest accounted for 88% of the eggs collected. A total of 12 lays were recorded from the filmed nests, with all laying events occurring in the evening and night hours between 17:30 and 20:30. Furthermore, in all the recorded lays, incubation began shortly after the eggs were laid. Since most eggs were collected during the subsequent early morning visit, there was an unknown period during which the female pre-incubated the egg, leading to an imprecise determination of the actual development time. For the purposes of this study, however, hour 0 was defined as the time the egg entered the incubator.

The initial weight of the eggs averaged 37.3±4.4 grams, with the maximum weight recorded as 46.3 grams and the minimum as 28.4 grams. An average mass reduction of 13.1% was observed from day 0 to day 18. The variation in embryo size was recorded for embryos that appeared normal (Figure 1).

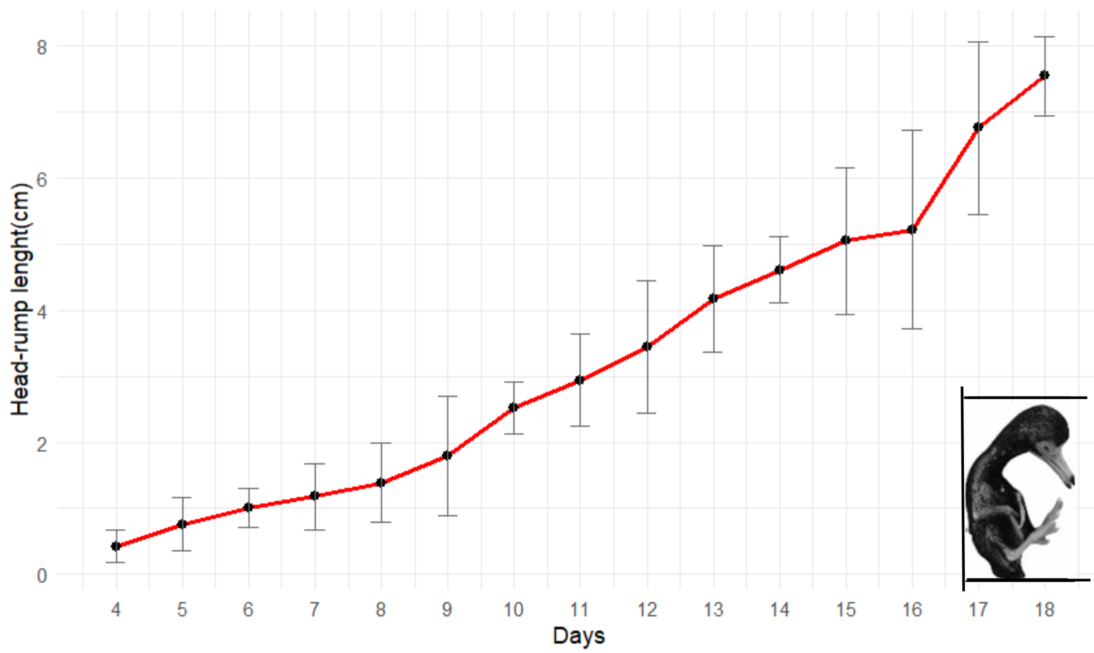

Figure 1 Daily mean head-to-rump growth of scarlet ibis from day 4 to day 18. Y axis: size variation (centimeters). X axis: days of incubation from day 4 to day 18.

3.1 Description of embryonic morphological variations in scarlet ibis

The following description focuses on the main external events that occur during the daily embryonic development of scarlet ibis, which can be easily assessed without any optical equipment. The complete development of the embryo can be divided in 3 phases, as described by Olea and Sandoval (2012) for semi-altricial type 2 domestic pigeon. Each day is also accompanied by the corresponding stages in precocial (HH) and altricial (ALT) species, which are used for comparative purposes and indicated in parentheses. The developmental chart of scarlet ibis along key features for each day of description are displayed in table 1 and figure 2.

Table 1. Comparation between morphological variations in embryo developmental charts for

different patterns of post-natal growth species.

Macroscopic characters Incubation Period	**Semi-altricial** 21 days	**Precocial** 21 days	**Altricial** 28 days	**Altricial** 14 days
Early phase. Day 0 to 7 (Stages 1 – 29)				
Limbs distinct	D3-4 (S19-22)	D 3-4 (S23)	D6-7 (S21-23)	D3-4 (S19)
Eye heavily pigmented	D5 (S22-24)	D3 (S22)	D6 (S23)	D5 (S27)
Digital plate in limbs	D5 (S22-24)	D4 (S24-25)	D8 (S25)	D4-5 (S25)
Upper beak protrusion	D6 (S25-27)	D5 (S28)	D9 (S27)	D5-6 (S28)
Both limbs digits visible	D6 (S25-27)	D 4-5 (S26)	D9 (S25-27)	D 5 (S26)
Mandibular process protrusion	D7 (S28-29)	D5-6 (S28)	D10 (S28)	D5-6 (S29)
Middle phase. Day 7 to 14 (Stage 30 to 38)				
Auditory meatus	D8 (S30-32)	D5-6 (S28)	-	D6-7 (S32)
Eyelids are first visible	D8 (S30-32)	D7 (S32)	D11 (S30)	D6 (S31)
Scleral papillae visible	D8 (S30-32)	D6-7 (S30)	-	D12 (S32)
Onset of neck elongation	D8 (S30-32)	D7-8 (S33)	-	-
Beak tips symmetrical	D9 (S33)	D7-8 (S32)	D11 (S30)	D7 (S33)
Alula	D10 (S34)	D7-8 (S33)	D12 (S32)	D7 (S33)
Egg tooth fully formed	D10 (S34)	D6-7 (S30)	D12 (S32)	D6-7 (S32)
First feather germs	D10 (S34)	D6-7 (S30)	D12 (S32)	D6-7 (S32)
Hallux	D10 (S34)	-	-	D7 (S34)
Scleral papillae completed	D10 (S34)	D7-8 (S33)	-	-
Web on radial margin	D10-11 (S35)	D7-8 (S33)	D12 (S32)	D7 (S33)
Nictitating membrane	D11 (S36)	D8 (S34)	D11 (S30)	D8 (S37)
Primordial of claws	D11-12 (S37)	D10 (S36)	D16 (S36-37)	D7-8 (S36)
Upper beak keratinization	D12 (S38)	-	D18 (S38)	-
Alar and tight feather germs	D12 (S38)	D7-8 (S36)	D14 (S35)	D8 (S37)
Late phase. Day 14 to hatching (Stages 39 to 46)				
Eyelids covering half of the lens	D14 (S40)	-	-	D8 (S37)
Scales in toes	D14 (S40)	D11 (S37)	D16 (S36-37)	D12 (S44)
Onset of toenails keratinization	D14 (S40)	D12 (S38)	D18 (S38)	D9-10 (S40)
Beak fully cornified	D15-16 (S41-42)	D13 (S39)	D23 (S44)	D12 (S44)
Toenails keratinization	D16 (S42)	D14 (S40)	D24 (S44)	-
Eyelids closed	D16 (S42)	D14 (S40)	D21 (S40)	D13 (S45)
Hatching muscle swelling	D16 (S42)	-	D22 (S40-44)	-
Head may be in the large pole of the egg	D16 (S42-44)	D14 (S40)	D24 (S44)	-
Head might be under the right wing	D16-17 (S42-44)	-	D24 (S44)	-

First column: the 21 days incubation period semi-altricial scarlet ibis with suggested possible stages for each developmental event. Second column: 21 days precocial chicken (Hamburger and Hamilton, 1951). Third column: 28 days altricial american kestrel (Pisenti, 2011). Fourth column: 14 days altricial zebra finch (Murray, 2013). **D:** Day of observation of the landmark (S):

Corresponding stage of development.

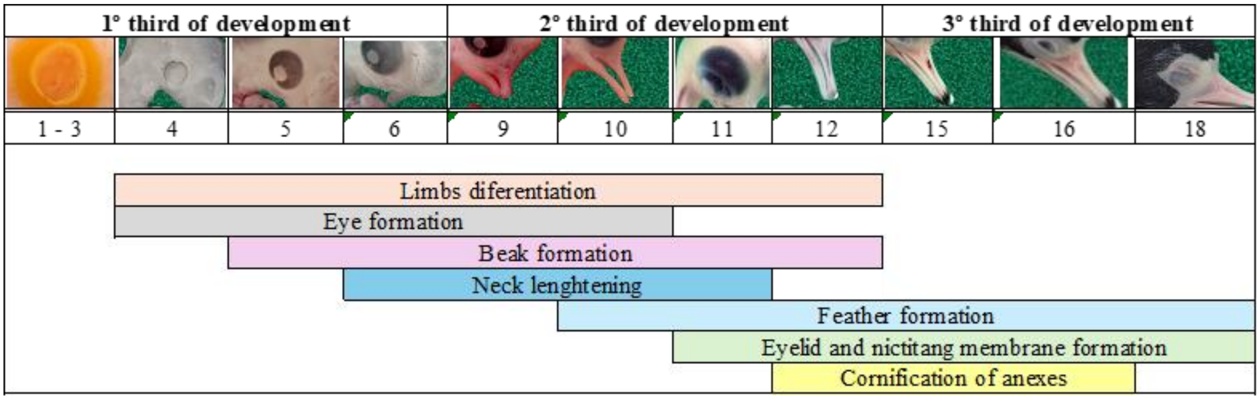

Figure 2 Chronology of key landmarks of scarlet ibis embryonic development. **Limbs differentiation:** from limb buds to fully formed autopodia. **Eye formation:** from the initial visibility to the completion of scleral papillae. **Beak formation:** from maxillary process protrusion to the final beak shape. **Neck elongation:** from the onset to final proportions. **Feather formation:** from initial buds on leg to full body coverage. **Eyelid and nictitating membrane:** from visible eyelid folds to closed eyelids. **Cornification of annexes:** from initial cornification to fully cornified beak and claws.

Early phase (stages 1-29) includes the initial development of the blastoderm and embryo formation, organization of the extraembryonic membranes, and the initial formation of the major organic systems. Diagnostic features are focused mostly on the initial development of blastoderm with the appearance of blood vessels, formation and growing of the embryo, development of eye, embryo position, initial development of limbs and beak and tail bud. The main characters of the first 3 days are better assessed by observing the changes from the yolk due to the small size and transparency of the embryo which difficult the manipulation and observation.

Day 1 (HH-ALT: Stage 6-11)

- Area pellucida forming around the primitive streak.
- No blood vessels yet.

Day 2 (HH-ALT: Stage 12-14)

- Extraembryonic membranes cover considerably part of the yolk surface.
- Blood ring is visible in the external side of the area pellucida that is now enlarged.
- Embryo distinctly visible.

Day 3 (HH: Stage 15-17 - ALT: Stages 12-18)

- Blood vessels grow in the yolk surface along the blastoderm oval.
- The embryo turns on its left side.
- The trunk is distinct.
- Tail bud extends along the axis.
- *Heart:* Ventricular loop of heart ventral to atrio-ventricular canal. Blood flowing with atrio-ventricular canal contraction.
- *Eyes:* Contour of eye is barely appreciable.

Day 4 (HH: Stage 18-21 - ALT: Stages 17-21)

- Embryo is on its left side with enlarged head and ventrally curved in a c- shape.
- The tail bud is at a right angle with the caudal axis.
- *Heart:* Heart now is a full circle.
- *Limbs:* Limb buds are enlarged and symmetrical.
- *Eyes:* Eye is faintly pigmented.

Day 5 (HH: Stages 22-25 - ALT: Stages 23-25)

- Trunk shortens significantly.

- *Limbs:* Digital plate on both limbs. Very faint initial distinction in forelimb digits. Toes not yet demarcated.
- *Eyes:* Eye heavily pigmented and lens clearly visible.

Day 6 (HH: Stage 25-28 - ALT: Stage 25-29)

- The chambers of the heart are no longer visible due to the thickening of pericardium walls.
- *Limbs*: Elbow and knee joint distinct. Digits of wing are clearly demarcated with an indication of a faint groove between them. Digits of hindlimb are visible but less than hindlimb digits.
- *Beak:* Upper beak is prominent. Mandibular process lengthened ventrally.

The middle phase (stages 30-38) was characterized mainly by the growth of limbs and the organization of the autopodium, the formation and growth of the beak, the lengthening of the neck, the formation of scleral papillae, and the development of integumentary annexes such as feather germs, eyelids and nictitating membrane.

Day 7 (HH: Stage 28-32 - ALT: Stage 27-29)

- *Limbs:* Digits of hindlimb are well demarcated with a faint groove between them. Alula is distinctly the shortest digit.
- *Beak:* Mandibular process protrudes forward and touches the base of the upper beak.

Day 8 (HH: Stage 28-33 - ALT: Stage 30-31)

- Neck is lengthened from day 7. Internal carotid artery is clearly visible on the left side.
- *Limbs:* Leg bends in knee joint.
- *Beak:* Mandible is about half length from upper beak.
- Eyelids folds are first visible.

- Auditory meatus is first visible.

Day 9 (HH: Stage 30-34 - ALT: Stage 30-33)

- *Limbs:* Second digit visible longer than the others. Legs longer than the tail bud.
- *Beak:* Upper and lower beak are almost equal size. Egg tooth partially visible.
- *Eyes:* Scleral papillae one on either side of choroid fissure.

Day 10 (HH: Stage 30-36 - ALT: Stage 32-34)

- *Limbs:* Digits of both limbs have lengthened. Alula separated from the wing tip. Tips of toes including hallux are discernible.
- *Beak:* Egg tooth distinct and slightly protruding. Beak is more pronounced with mandible slightly longer than maxilla.
- *Eyes:* Scleral papillae complete except for ventral point.
- *Feather germs* Feather germs on either side of the spinal cord and in the femoral region of the leg.

Day 11 (HH: Stage 33-35 - ALT: Stages 34-37)

- Cloaca distinctly protruded.
- *Limbs:* Toes well separated. Lengthening of digits continues. Web on radial margin becomes discernible.
- *Eyes:* Nictitating membrane is clearly visible. Eyelids has reached almost to scleral papillae and have extended towards the beak.
- *Feather germs*: Feather germs between eyes, head, neck, trunk, and next to ventral midline close to sternum. Primordia of flight feathers and tail buds are just visible.
- *Neck:* Final form and size relative to head.

Day 12 (HH: Stage 35-39 - ALT: Stages 35-40)

- *Limbs:* Phalanges in toes are distinct. Primordia of claws is discernible.
- *Beak:* Tip of upper beak becomes darker as keratinization starts.
- *Eyes:* Eyelids start forming an ellipse outgrowing the eye and covering scleral papillae. Nictitating membrane covers the anteriormost scleral papilla.
- *Feather germs*: A few dorsal, axillary and tail black feather primordia are elongated. Primary feather buds on manus are visible. Feather buds on tights.

Day 13 (HH: Stages 37 - ALT: Stages 35-37)

- *Limbs:* Toepads are visible. First digit toenails curved ventrally. *Eyes:* Lower eyelid starts covering the cornea. *Feathers*: Black feather primordia on the wing are visible. Feathers of the dorsal trunk, femoral region and base of the wing have elongated.

Day 14 (HH: Stage 37-38 - ALT: Stages 38-40)

- *Limbs:* Claws tips keratinization. Very discrete scales in the dorsal aspect of the toes tarsus may be present.
- *Beak:* Beak keratinization continues, and lower beak tip becomes darker.
- *Feathers:* Few black feather primordia on upper and lower eyelids. Black feathers are covering the dorsal and ventral aspect of the body, neck and head, contrasting with much shorter wing coverts and thigh feathers.

The late phase (stages 39-46) shows the overall embryo growth, the final organization of the feather distribution pattern and growth of feather germs, keratinization of beak and claws, formation of scales in legs, growth of eyelids, and in the last stages, growth of hatching muscle as well as final consumption of the yolk, and hatching.

Day 15 (HH: Stage 38 - ALT: Stage 38)

- Meconium may be present.
- *Beak:* Keratinization of the beak advances as both upper and lower beak tips are markedly darker.
- *Eyes:* Upper eyelid is covered with black feather primordia. Few rows of black feather primordia in the lower eyelid. Opening between the lids is reduced.
- *Feathers:* Wing coverts and thigh feathers are growing.

Day 16 (HH: Stage 37-40 - ALT: Stage 40-44)

- Head may be near the air cell and below the right arm. Amniotic fluid may starts decreasing.
- *Neck:* Hatching muscle starting to swell.
- *Limbs:* Keratinization of claws is completed including alula claw.
- *Eyes:* Eyes are almost closed.
- *Feathers:* Black feathers are filamentous over the entire body excluding manus.

Day 17 (HH: Stage 42 - ALT: Stage 38-45)

- Head may be near the air cell
- *Limbs:* Claws are perpendicular to the toes.
- *Beak:* Beak appears to be fully cornified (PI 44). Dark mark of the tip of the beak is noticeable enlarging towards the head.
- *Eyes:* Eyelids fully closed.
- *Feathers:* Feathers of manus are filamentous and black.
- *Neck:* Hatching muscle swelling is more marked.

Day 18 (HH-ALT: Stage 43)

- Head may be near the air cell and below the right arm.

- Maximum edema of the hatching muscle.
- *Beak:* Dark mark of the tip of the beak is advancing posterior.

Day 19 (HH-ALT: Stage 44)

- Internal membrane may be pipped.
- Head under the right arm and beak pointing to the air cell.

Day 20 (HH-ALT: Stage 45)

- Small circled crack in the middle of the large pole tip and equator may be present.
- Yolk may be completely incorporated into the abdominal cavity.

Day 21 (HH-ALT: Stage 46)

- Neonate with closed eyes and low mobility.
- Yolk is completely incorporated into the abdominal cavity.

4 Discussion

This is the first study to describe the development of a semi-altricial species with the same developmental period as the domestic chicken, allowing for a comparison of the chronology of developmental landmarks and potential heterochronies between two different post-natal growth patterns. Previous descriptions have been made with species having different incubation periods, which reduces the accuracy of comparisons (Herbert, 1967; Olea and Sandoval, 2012).

Given that embryo development is a continuous process, studies describing it often use a large number of embryos to estimate the absolute incubation time. This approach aims to describe every single developmental stage, accounting for the natural variation between embryos at the same incubation age (Murray, 2013). However, limitations related to the conservation status of studied species and availability of embryos have led many studies to describe only relative stages as a general reference for the species, due to insufficient embryos for complete descriptions (Browne, 2006; Vaz Guida, 2018). Nonetheless, these partial descriptions are highly valuable for accurate aging and detection of abnormalities in specific embryo development, which can only be fully assessed when compared with the normal development of the same species (Pisenti et al., 2001; Cordero and Werneburg, 2022).

The pre-incubation of eggs by parents before collection was related by Pisenti (2001) as a confounding factor, particularly in early development. Additionally, the use of more rudimentary incubators leads to less uniform environmental conditions, which can cause variations in the development speed of embryos of the same age (Pereira et al., 2021). Therefore, although we describe morphological variations for each day of development, it is important to note that the described stage represents just one of the possible stages that can occur within a 24-hour period. This approach is intended to facilitate practical diagnostics as required in incubation facilities.

Table 2. Selected macroscopic features of daily embryo development of scarlet ibis. Descriptions from day 1 to day 18 for practical diagnostic identification in incubation facilities. Days 19 to 21 exhibited only changes in embryo positioning and are not presented here.

Figure 3 Daily morphological main events in scarlet ibis. The figure displays the embryo morphological variations day-to-day from day 1 to day 18.

Our description aimed to establish developmental landmarks that can be assessed through direct macroscopic observation and can be applicable across different levels of infrastructure. Several landmarks identified for other species are only relevant to live embryos, contrasting with artificial incubation routine. In these settings, embryo development failures are often discovered days after the embryo has died (Pereira et al., 2021). This delay makes it challenging the assessment of certain characteristics in autolyzed tissue and, consequently, to determine the age of the deceased embryo (Pisenti et al., 2001; Kasielke, 2020).

Nuñez-León et al. (2001) identified limbs and beak growth as characteristics that could potentially exhibit heterochronic variations between species, while Cordero and Wernerburg (2022) highlighted eyelids features as well. In the mid phase of development, these features were confirmed in scarlet ibis, with notable variations in limb development occurring between days 4 and 11, and of beak development between days 5 and 10 **(**Figure 3). Additionally, we observed that neck lengthening, between days 7 and 11, serves as a sensitive marker for scarlet ibis, as does the onset of beak keratinization, marked by characteristic dark pigmentation at the tip, between days 12 and 16. Positioning of the embryo showed high variability between days 15 and 18, reducing its diagnostic utility.

Although there was a relatively good concordance between the stages of development in the scarlet ibis and those related by Hamburger and Hamilton (1951) on precocial chickens, the timing of the appearance of some of the key diagnostic landmarks differed of up to 4 days, despite both species having the same incubation period. Therefore, we suggest that the embryonic development of precocial species can only serve as a general reference for the sequence of developmental events, but not for accurately determining the age of altricial and semi-altricial embryos. This finding contrasts with Handbidge and Fox's (1996) suggestion that a reliable estimate of embryo age can be obtained by comparing embryos across species with similar incubation periods, regardless of their post-natal growth patterns (Table 2 - Figure 3).

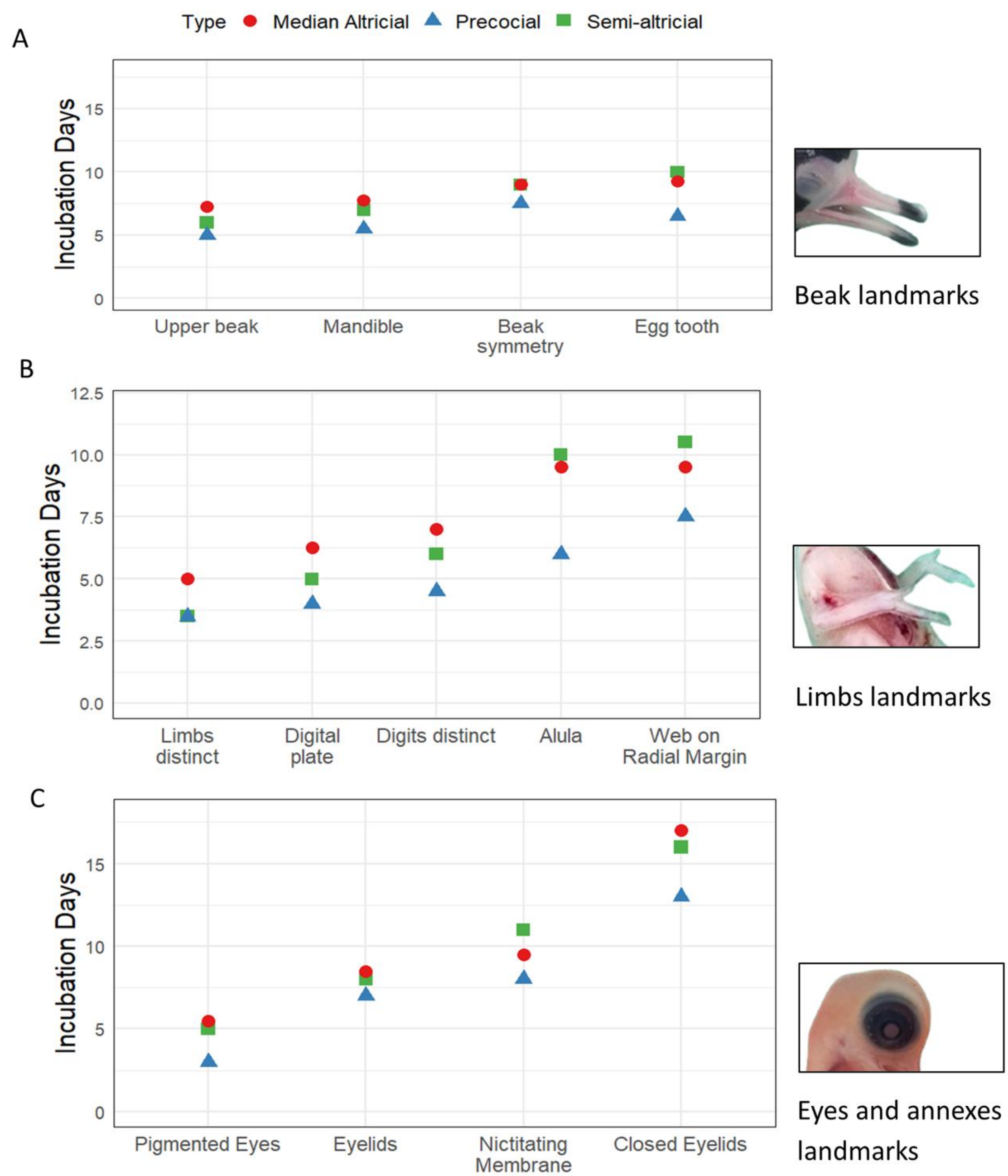

Figure 4 Heterochronies on embryo developmental landmarks across different post-natal growth patterns. The figure illustrates the timing of appearance for selected landmarks from table 1, on the semi-altricial scarlet ibis, comparing with the same landmarks in altricial chicken and the median

timing for two altricial species, with 14 and 28 days of incubation period. A) Beak. B) Limbs. C) Eyes landmarks.

Conversely, the embryonic development of altricial species with both shorter and longer incubation periods (Pisenti et al., 2001; Murray et al., 2013), was relatively consistent with the development observed in the semi-altricial scarlet ibis. During early development, the scarlet ibis embryo showed some synchronization with the zebra finch but was approximately 2-3 days behind the American kestrel. In contrast, during the mid and late phase of embryonic development, key landmarks in the scarlet ibis occurred relatively near to the median of the days of the two altricial species. This suggests that the timing of developmental landmarks in semi-altricial species may resemble that of altricial species with the same incubation period.

5 Conclusion

The macroscopic descriptions obtained in this study reveal significant variations that support the development of a species-specific chart for the embryonic development of the scarlet ibis. This chart may also have applications for other ibises, given the shared distinctive features among several members of this family. Employing precocial charts to evaluate the development of altricial and semi-altricial species can lead to inaccuracies in age determination. Notably, the development of the semi-altricial scarlet ibis may closely resemble that of altricial species with equivalent incubation periods. Ultimately, this research will serve as a valuable tool for diagnosing incubation issues in the scarlet ibis and will enhance field studies related to this species.

Conflicts of Interest

The authors declare no conflicts of interest.

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CAPÍTULO II

MANAGEMENT AND REPRODUCTIVE PARAMETERS OF A CAPTIVE SCARLET IBIS (Eudocimus ruber) POPULATION AT LOW LATITUDE

Management and reproductive parameters of a captive scarlet ibis (*Eudocimus ruber*) population at low latitude

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Abstract
Effective captive breeding programs are essential for conserving threatened avian species. This study aimed to describe the breeding performance of a captive population of scarlet ibis over a three-year period (2022, 2023, and 2024). The breeding season varied between July and January over the three years. Both males and females began forming breeding pairs as early as two years old, with the oldest pairing ages being 20 years for males and 12 years for females. Higher enclosure densities appeared to improve laying rates and encourage earlier laying, while a 1:1 male-to-female ratio facilitated effective pair formation with minimal aggression. Egg removal led to an average of 7.6 ± 2.7 replacement lays per female. Advancement of the laying season was significantly associated with increased initial egg weight ($p < 0.01$). The median daily weight loss during incubation was 0.74% (tau = 0.5), while the mean weight loss on day 21 was 19 ± 2%. The findings from this study are relevant to enhance the efficiency of breeding programs for the species and may inform breeding strategies for other species within the Threskiornithidae family.
Key words: Birds, breeding, incubation, ibis, conservation.

1 Introduction

The scarlet ibis (*Eudocimus ruber*) is a tropical bird with distribution throughout northern South America, mainly occurring in coastal and river ecosystems. Its population is continually declining (Birdlife international, 2022) and is endangered or already extinct in several regions of its original range (Sao Paulo, 2009; EBIRD 2015). In the wild the scarlet ibis breeds in groups of tens to hundreds of individuals with a marked synchronicity, displaying nesting pulses with variable laying and hatching rates between them (Olmos 2001).

Although the species displays breeding activity in zoological collections, its reproductive management, as in several other species, remains underdeveloped, with no standardized protocols available (Miranda et al., 2021). Insufficient data to support management decisions is seen as a major barrier to achieving successful breeding outcomes in captive wild birds. This issue is particularly critical for threatened species, where the small number of individuals makes successful reproduction highly dependent on precise and informed decision making (Smith et al. 2011; Edwards et al., 2021).

To enhance the success of captive breeding programs, researchers have increasingly looked to industrial poultry farming as a model, due to its advanced technical practices that can potentially be adapted for wild bird species (Brown, Keefer, and Songsasen, 2019; Pereira et al., 2021). Key aspects such as breeder selection, egg management, and the use of productivity indexes are widely applied in industrial poultry and are central to their high reproductive efficiency. However, these practices are still largely unincorporated into the captive breeding of many wild species, including the scarlet ibis.

The basic breeder selection begins by defining key parameters such as fertility and hatchability which is a priority index in decision-making within any effective breeding program. These indicators are influenced by factors such as genetics, breeder age, incubation conditions, and egg characteristics, all of which must be evaluated and managed on a species-specific basis to optimize reproductive success and enhance fledgling output over time (Kingori, 2011; Salamon, 2020; Pereira et al., 2021).

Regarding egg management, egg removal is a widely used technique adapted from poultry production to enhance chick output. By removing eggs shortly after laying, females are stimulated to produce additional clutches, as this practice prevents the prolactin-mediated negative feedback on reproductive hormones (Riou, 2010; Angelier et al., 2013). While this strategy has been successfully adopted in the breeding of threatened bird species, the long-term effects of repeated clutch induction on female health and future reproductive success remain unclear (Boyce et al., 2005; Rufino et al., 2014; Pereira et al., 2021).

Among waterbirds, the extensive efforts dedicated to the endangered whooping crane serve as a strong example of applying zootechnical principles to conservation breeding (Smith et al., 2011). Once critically endangered, the species has benefited from research focused on factors affecting fertility and hatchability of captive breeders (Smith, 2011; Brown, Keefer, & Songsasen, 2019) and the strategic trade-offs between egg removal, incubation by foster crane species and artificial incubation to increase chick output (Boyce et al., 2005; Edwards et al., 2022). These approaches have contributed significantly to the species' population recovery over recent decades.

Regarding the Threskiornithidae family, although several aspects differ significantly across species, many physiological and behavioral traits are sufficiently similar to possibly allow for cross-species extrapolation (Archibald et al., 1980). A notable example is how artificial incubation and hand-rearing basic techniques developed for white, glossy, and scarlet ibises have informed conservation breeding practices for the endangered Japanese crested ibis (*Nipponia nippon*) (Archibald et al., 1980). These methods are now also being adapted to support the breeding management of the critically endangered white-shouldered ibis (*Pseudibis davisoni*) (Woesner et al., 2021).

Therefore, this study aimed to describe basic reproductive management—such as breeder selection, egg selection, and egg management practices—for a captive breeding program of the scarlet ibis, based on established poultry practices. The data generated in this study are expected to support informed decision-making in scarlet ibis captive breeding efforts and may also serve as a model for breeding programs of other endangered species within the Threskiornithidae family, particularly those with similar reproductive traits that allow for extrapolation.

2 Methods

2.1 Ethical aspects

All experimental procedures in this work plan were approved by the UFPA Animal Research Ethics Committee (CEUA Nº 7584300323).

2.2 Location and period of study

The study was conducted in the Mangal das Garças Zoo Park located in the city of Belém, state of Pará-Brazil, 1°27’48.9”S 48°30’17.8”W. AM-SEMAS Nº 544879. The study was conducted during the breeding seasons from 2022, 2023 and 2024. The date of the first egg laid until the date of the last egg in the series was considered the total length of each breeding season. Recorded temperature ranges during these periods were 22.9–32.3°C (2022), 23.5–33.7°C (2023), and 24.4–33.6°C (2024). As expected for a low-latitude location, daylight hours exhibited minimal variation across the three years.

2.3 Study animals

The study was conducted with eggs laid by a group of 40 scarlet ibis (20 males and 20 females) previously sexed by molecular tests, aged between 2 and 20 years old, born in captivity in the zoo's species breeding program. The enclosures have concrete and sand substrate, artificial lake, natural perches, and natural light in the morning and afternoon. Artificial nests were installed after observing courtship behavior, being made of wood and plastic mesh at the bottom in order to reduce the risk of broken eggs. A video recording from a single camera positioned inside each enclosure partially captured the nesting and breeding behavior of the pairs in year 3. The animals were fed with species-specific feed (Flamingo/guará Fl32 Megazoo ®, São Paulo, Brazil) containing 32% protein, 5% crude fiber, and 7% ether extract, among other components, in the amount of 50g/kg/animal once per day with scheduled supplements with a multivitamin complex + powdered probiotics (Organew - Vetnil ®) and mealworms.

2.4 Egg management

The nests were inspected 7 times a day from 06:00 to 18:00. The eggs were removed from the nest as soon as possible and the couple were identified according to prior marking in the matrices. The eggs were taken to the incubatory, sanitized with 70% alcohol and screened for shell defects. Only eggs with cracks in the shell were discarded. Each egg was then weighed using an electronic scale with a sensitivity of 0.1g. Measurements of maximum length and maximum width were taken with a manual caliper. The shape index was determined by the formula described by Yakubu et al. (2008):

$$\text{Shape index} = \frac{\text{Egg width (cm)} \times 1000}{\text{Egg length (cm)}}$$

2.5 Incubation

The egg was identified with a pencil and placed in a digital incubator, with automatic turning programmed every two hours (IP 130 PS, Premium Ambiental Ltda., Belo Horizonte, Minas Gerais, Brazil). The temperature and humidity were programmed at 37.5°C and 60% respectively as recorded by García et al., (2014) for the species.

We weighed the eggs on days 0, 6, 12 and 19, when they were separated for hatching. We performed ovoscopy on each of these days to monitor embryo development and infertile eggs or dead embryos were discarded following embryo necropsy. Eggs showing no membrane development during candling were opened to assess fertility.

Before hatching, each egg was weighed and transferred to another incubator of the same model, conditioned as a hatching unit with a temperature of 37°C and humidity of 65% (García et al., 2014), where after hatching the chick was kept for 2 days.

2.6 Statistics

Production per female (%) was estimated as the ratio between the number of eggs laid and the duration of the reproductive period. Fertility was defined as the ratio between the total number of eggs laid and the total number of fertile eggs. Hatchability was defined as the ratio between the total number of fertile eggs and the total number of hatched eggs. The hatching rate was defined as the ratio between the total number of incubated eggs and the total number of hatched eggs. Statistical analyses were processed using the R statistical program correlating the data obtained as follows:

• Influence of the age of the female on the laying rate (total number of eggs laid): Mixed-effects model

• Influence of the age of the female on fertility (number of fertile eggs in the total number of eggs): Generalized linear mixed model.

• Influence of the age of the male on fertility (number of fertile eggs in the total number of eggs): Generalized linear mixed model.

• Influence of the age of the female on hatchability (number of hatched eggs in the total number of fertile eggs): Generalized linear mixed model.

• Influence of the shape index on the hatchability: Logistic regression.

• Influence of the age of the breeder on the shape index (egg size): Generalized linear mixed model.

• Influence of egg weight loss during incubation on the hatchability: Quantile regression.

• Influence of the age of the male and female on the laying period (time segment in which the eggs are laid during the reproductive season): Linear regression.

• Influence of the laying period on the initial egg weight: Mixed-effects model.

3 Results and discussion

Relevant indicators of the breeding season are detailed in **Supplementary material**. The onset of the laying period varied between June and August over the three years, while the end occurred between August and January (**Figure 1**). The laying density during these three years primarily followed two patterns. The first and more common pattern, as described by Olmos (2001), showed two peaks of laying: one from June to August and another from November to January of the following year, with the latter being less pronounced. The second pattern was observed only in the first year and consisted of a single peak of laying between August and December. This difference in patterns may be attributed to the initial adaptation of the pairs to their enclosures and mating conditions. In 2023, there was a significant drop in laying density due to major renovations at the zoo. The stress caused by this sensory stimulus may have contributed to the premature closure of the laying period.

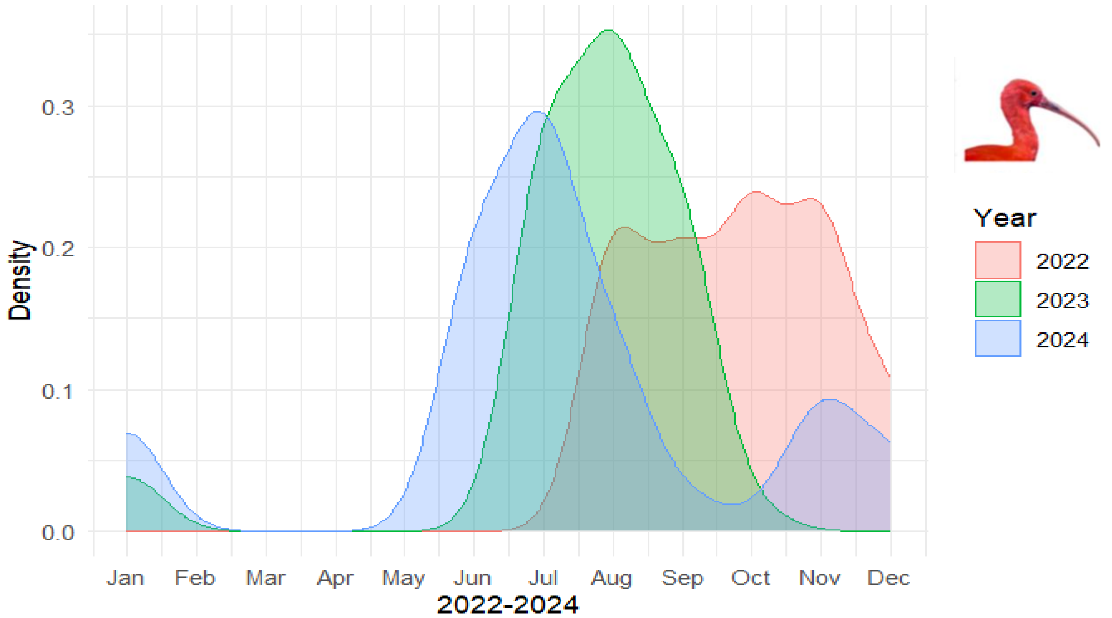

Figure 1 Monthly Egg-Laying Distribution Over a Three-Year Period. This density plot depicts the distribution of egg-laying events for scarlet ibis across three consecutive years: 2022 (red), 2023 (green), and 2024 (blue). The x-axis shows the months from January to December, while the y-axis represents the density of egg-laying occurrences, highlighting seasonal patterns over time.

There was a positive correlation between enclosure density and the number of eggs laid. This can be explained by the nesting behavior described by Olmos (2013), which indicates that scarlet ibises prefer to build their nests near one another. This strategy helps reduce the likelihood of predation on their nests within a densely packed group of nests.

Early morning visits to the nests accounted for 88% of the eggs collected. All recorded egg-laying events occurred in the evening and night hours, specifically between 17:30 and 20:30. To our knowledge, this is the first time these laying hours have been documented for the species. Additionally, in all recorded layings, incubation began shortly after the eggs were laid, typically initiated by the female. This is crucial for the storage of eggs of the species, as gastrulation of the embryo occurs within the first four hours after laying. Storing eggs at low temperatures beyond this period can lead to increased early mortality (Khan et al., 2014).

The scarlet ibis exhibited low selectivity toward artificial nest types, which is consistent with descriptions by Olmos (2001), who attributed this behavior to the wide variety of tree species used for nesting in their natural mangrove habitats. The regular provision of nesting materials strongly stimulated nest-building behavior and significantly reduced egg breakage, aligning with findings reported by Xi et al. (2002) and Huygue et al. (2023) in studies on the crested ibis.

Egg withdrawal proved to be highly effective in increasing the laying rate for the species, resulting in a maximum of 18 eggs laid following repeated removals, and an overall average of 7.6 ± 2.7 eggs across the 3 years period. These results are consistent with those reported by Pereira et al., in 2021. Additionally, Olmos (2001) documented a high egg loss rates in wild scarlet ibis populations, which are often followed by repeated laying during periods of food abundance. This natural tendency for clutch replacement may help explain the high responsiveness to egg removal in captivity. However, as of August 2023, a maximum limit of eight removed eggs has been established to safeguard animal welfare.

The first strategy implemented to prevent further laying involved leaving the egg in the nest with the parents. However, this approach led to a higher incidence of soiled and broken eggs, consistent with findings in crested ibises (Nipponia nippon) reported by Xi et al. (2014). A second strategy, which involved replacing the removed egg with a dummy egg, aimed to encourage continued brooding behavior. Although some pairs still laid a second clutch following the introduction of the fake egg, this method was largely effective in suppressing additional laying and contributed to the observed reduction in egg numbers during the 2023 and 2024 breeding seasons.

Pereira et al., (2021) emphasize the need for timely egg removal after laying in order to reduce brood stimulation, which can decrease or halt further egg laying. In the case of the scarlet ibis, nocturnal lays were collected the following morning, several hours later, with seemingly little effect on most breeding pairs. However, this factor may contribute to the reduced rate of egg laying and the frequent changes in pairs within enclosure 3. Further research is needed, including the examination of other environmental cues, such as luminosity, to better understand the variations between enclosures.

Nevertheless, the records from enclosures 1 and 2 suggest that a margin of 12 hours between egg laying and egg withdrawal is acceptable to maintain laying activity for the species. Detailed data on egg replacement and the monogamy of pairs are presented in **Table 1**.

Table 1 Mean values of monogamy among pairs in a single breeding season and replacement lays by a female after withdrawal.

	E1	E2	E3	Mean 3 years period
Mean monogamy**	82.2 ± 16.8	87.8 ± 10.7	78 ± 19.6	82.6 ± 4.9
Mean laying replaces after removal	8.1 ± 3.5	9.9 ± 4.5	4.7 ± 3.3	7.6 ± 2.7

* Mean values for the three enclosures and for each of the 3 years.

**Includes birds removed from enclosure due to health issues.

Some authors caution that egg production is both nutrient and energy demanding process for females (Monaghan & Nager, 1997; Edwards et al., 2022). However, existing studies often fail to clearly separate the energetic costs of egg production from those associated with brooding and chick-rearing. As a result, it remains difficult to isolate and evaluate the specific health impacts of repeated egg laying, independent of the additional energy expenditure required for offspring production (Milonoff & Paananen, 1993; Monaghan et al., 1995).

Nonetheless, research suggests that when breeders are in good physical condition and receive adequate nutritional supplementation, their fitness is unlikely to be adversely affected by the production of additional clutches (Heaney & Monaghan, 1996; Rufino et al., 2014). Still, despite the association with increased egg loss, permitting brooding and chick-rearing remains essential for avian welfare, as these behaviors constitute a fundamental component of the natural reproductive cycle.

Furthermore, care must be taken to avoid the inadvertent selection of individuals with exceptionally high reproductive output, as this can lead to a long-term reduction in the genetic diversity of the species. Effective management of this risk requires a robust record-keeping system to track breeder lineage, along with strategies such as the equalization of family sizes, which prioritize the breeding of individuals that contribute to maintaining overall heterozygosity within the population (Williams & Hoffman, 2009).

The youngest age at which both male and female scarlet ibises begin forming couples and laying eggs is 2 years. Sorato and Kotrschal (2006) reported that northern bald ibises (Geronticus eremita) reach sexual maturity within the first 3 years, while Groot et al. (2024) suggest that white-shouldered ibises (Pseudibis davisoni) mature even earlier.

The oldest individuals in the experiment—20-year-old males and 12-year-old females—successfully formed pairs and laid eggs, exhibiting hatching rates comparable to those of younger birds. The female had a significant positive effect on fertility, with some females showing consistently higher fertility rates than others ($p<0.05$; **Table 2**). However, female age itself did not significantly influence fertility, which may be due to the possibility that the oldest individuals in the breeding cohort have not yet reached reproductive senescence. This is consistent with reports of the species reaching up to 33 years of age in captivity (Garcia, 2014).

Table 2 Correlation of data obtained during the breeding seasons of 2022, 2023 and 2024.

Correlation of data	Result
Female age / Laying rate	NS
Female age / Fertility	NS
Female age / Hatchability	S (P<0,05)
Shape index / Hatchability	NS
Egg mass loss / Hatchability	(95% CI= 0.72, 0.76)
Male age / Laying period	NS
Female age / Laying period	NS
Egg weight / Laying period	S (P<0,01)

Four correlation factors showed significance: The female age is a predictor of hatchability (P<0,05). The median weight loss during incubation increases by approximately 0,74 percentage points for every day (0,72 to 0,76 - 95% confidence interval). The advancement of the laying season period is a predictor of the initial egg weight (P<0,01).

Age had little overall effect on laying rate, with the only notable pattern in scarlet ibises resembling that reported by Yu et al. (2014) for crested ibises: individuals aged 2–3 years and those over 10 years tend to produce smaller clutch sizes compared to birds aged between 4 and 10 years.

Furthermore, in contrast to patterns reported in other wild bird species (Brand, 2012; Pereira et al., 2021), age also significantly affected hatchability (P<0,05; **Figure 2**), but with unexpectedly high success in the youngest (2-5 years) and oldest females (9-12 years), and lower values in middle-aged cohorts (6–8 years). This discrepancy may reflect either sampling bias (middle-aged females contributed 54,6% of fertile eggs, amplifying stochastic effects) or life-history trade-offs, where younger females prioritize egg quality over quantity and older individuals benefit from experience. Future studies should consider clutch size and individual reproductive history to clarify these mechanisms.

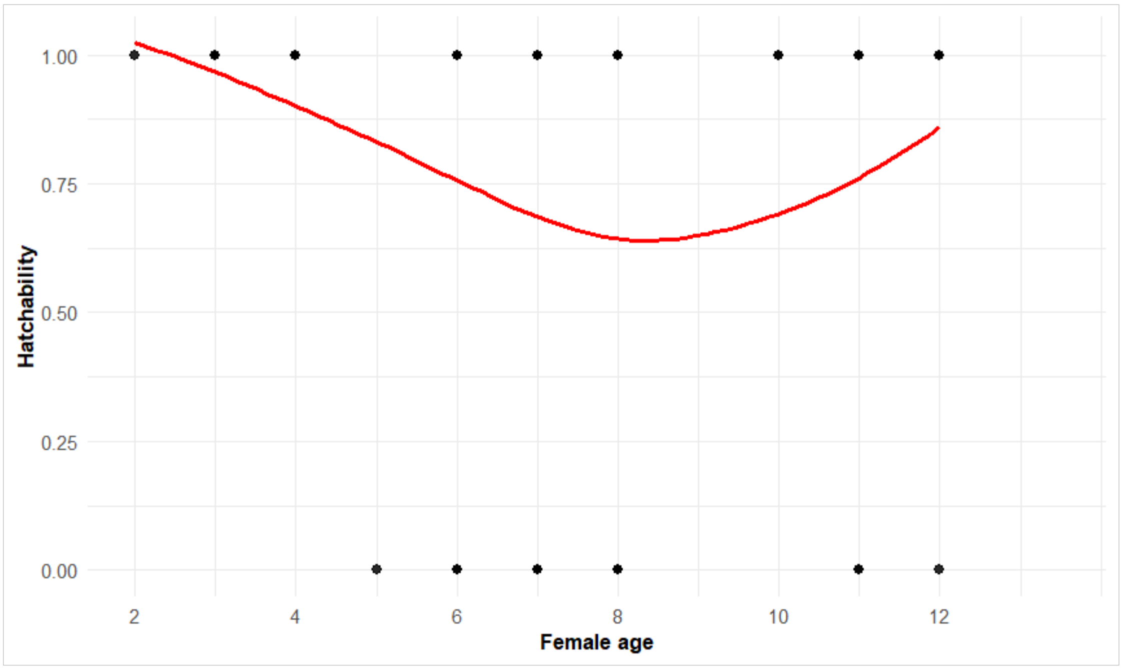

Figure 2 Relationship between female age and hatchability in Scarlet ibis. Black dots represent observed hatchability values (0 or 1), while the red curve shows the predicted probabilities from a generalized linear mixed model with a binomial distribution, including linear and quadratic terms of female age. The model indicated a significant non-linear effect of female age on hatchability (P<0,05), suggesting that both very young and older females tend to have higher hatching success compared to middle-aged individuals.

The advancement of the laying season over the three-year period had a significant positive effect on initial egg weight ($p < 0.01$; Figure 3). Although this result contrasts with some previous studies in wild and domestic species (Nager et al., 1999; Salamon & Kent, 2013), the progressive increase in egg weight observed in scarlet ibises more closely resembles findings from non-seasonal laying hens maintained under controlled light conditions (Alvarez & Hocking, 2012; Salamon & Kent, 2013).

This pattern suggests that, like commercial laying hens, scarlet ibises may rely more on exogenous energy sources for egg production rather than on endogenous nutrient reserves. As a result, increased nutritional supplementation during the laying period—especially in the context of repeated egg removal—may have contributed to the observed increase in egg weight.

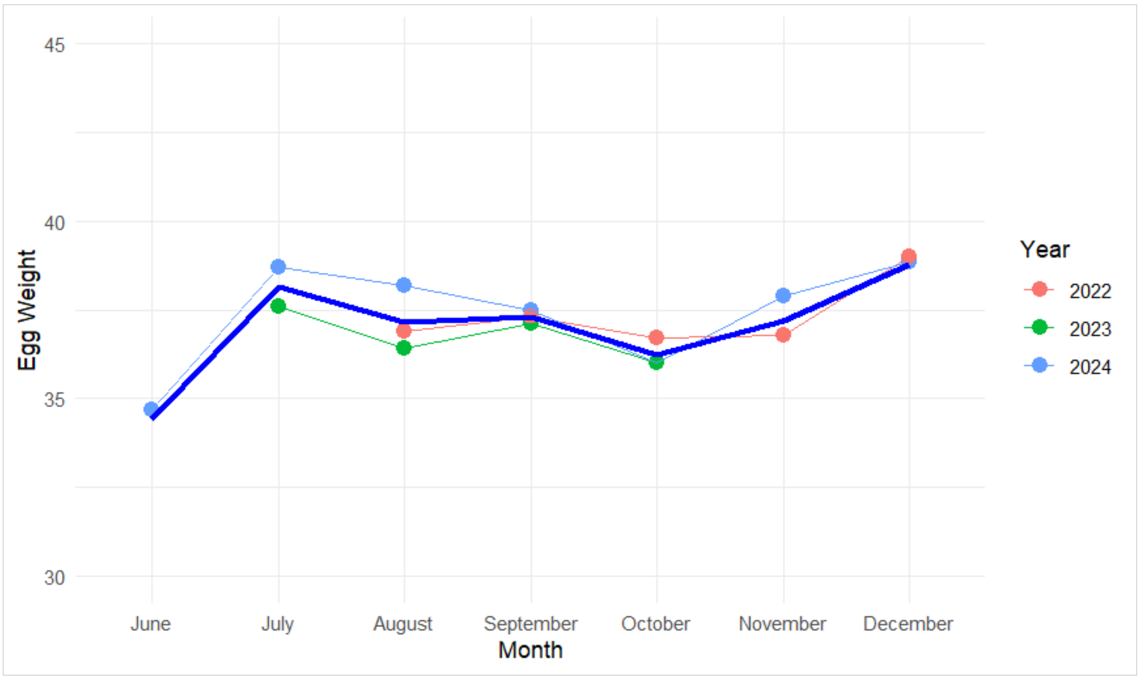

Figure 3 Egg Weight by Month – Mixed Effects Model. This figure presents a positive correlation (p<0,01) between month of laying and the egg weight, based on data collected over three years (2022, 2023, and 2024). Mean egg weights (points and connecting lines for each year) are plotted, while the blue dashed line represents the fixed-effects prediction from a linear mixed-effects model (with Month as a fixed effect and Year as a random effect). The y-axis is the weight in grams. On the x-axis illustrate the overall trend in egg weight across the laying seasons.

The median daily weight loss during incubation was 0.74% (τ = 0.5; Figure 3), and the mean total weight loss by day 21 was 19 ± 2%. Rahn and Ar (1980) reported an average egg mass loss of 15% prior to external pipping across 81 bird species, though individual species exhibited a broader range of losses, from 10% to 23% (Zicus et al., 2004). These variations are likely influenced by environmental factors such as temperature and humidity during incubation.

In artificial incubation, these environmental conditions are primarily determined by the quality of the incubator. Unlike advanced industrial models, domestic incubators typically maintain constant temperature and humidity throughout the incubation period. However, as the embryo develops and begins producing more metabolic heat, slightly reduced incubation temperatures and higher humidity levels may be more appropriate. Inadequate adjustment to these changing needs may result in deviations from the expected mean egg mass loss. Nonetheless, our observations indicate that values within the recorded confidence interval remain acceptable for successful hatching of scarlet ibis eggs.

Egg size or weight did not influence hatchability in this study. While hatchability is a critical first step in reproductive success, chick survival after hatching is equally important. Several studies have examined the effect of egg weight on chick viability in both wild (Blomqvist et al., 1997; Krist, 2010) and domestic species (Wilson, 2007; Iqbal et al., 2016; Yerpes et al., 2020), with mixed results. In the case of the scarlet ibis, although egg weight did not seem to affect hatchability or chick survival at first glance, further targeted studies are needed to determine whether egg weight may influence post-hatch traits such as weight gain and immune function.

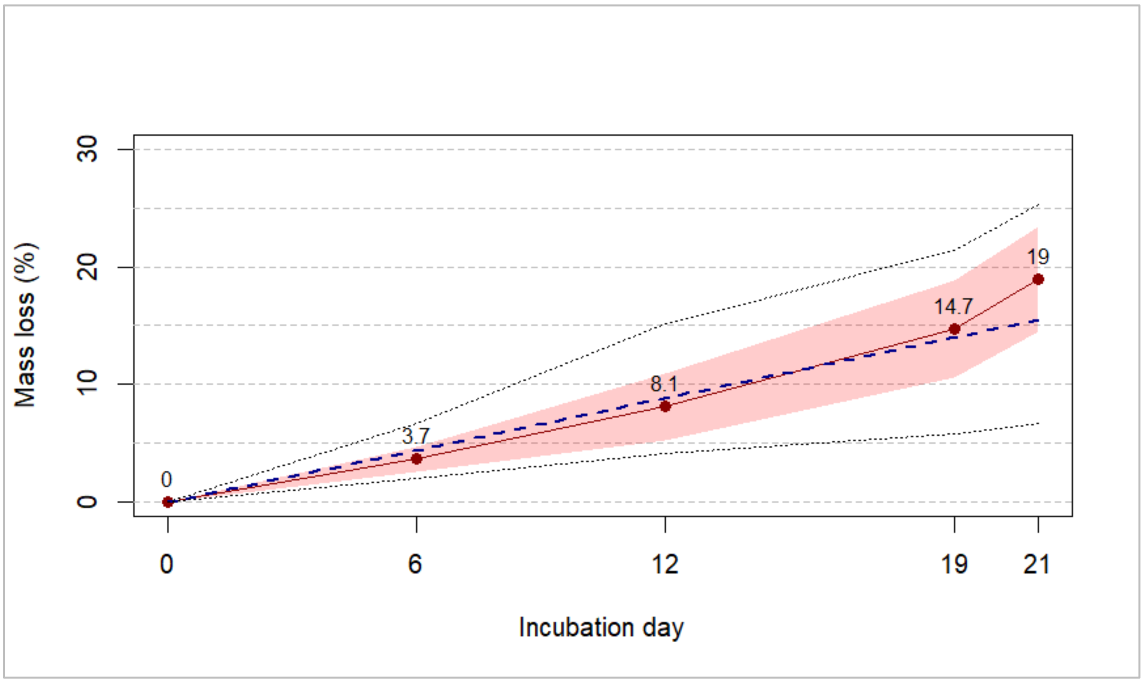

Figure 3 Quantile regression of egg weight loss leading to successful hatching: This figure displays a quantile regression (blue line) with the shaded region representing its confidence interval. The red points indicate the mean weight loss at each incubation day, while the black lines denote the maximum and minimum percentages of egg weight loss compatible with hatching.

Optimizing captive breeding programs could benefit several scarlet ibis populations worldwide by enhancing genetic diversity and supporting species conservation. Such efforts may also strengthen reintroduction programs, especially given existing reports of successful adaptation of captive-bred scarlet ibises to the wild—evidence that their native ecosystems can still support survival (Olmos, 2015). It is important to emphasize that captive breeding and release should be viewed only as short-term, complementary actions within a broader, long-term conservation strategy for the species (Snyder et al., 1996; González et al., 2020).

Conclusion

Several aspects of scarlet ibis reproductive biology were described for the first time in this study, providing a detailed and operational basis to guide decision-making in breeder selection and artificial incubation.

Given that several observations of this study are consistent with zootechnical parameters observed in domestic species under intensive production systems, the methodologies and reproductive responses documented in scarlet ibis breeders may provide a valuable framework for application in conservation strategies targeting other ibis species as well. Such results have the potential to broaden the operational scope of ex situ management, increase the predictability of breeding performance, and enhance overall productivity through the implementation of standardized protocols that integrate both animal welfare and reproductive efficiency.

Ethics statement

This study was conducted in accordance with the Ethics and Animal Welfare Committee of the Federal Rural University of the Amazon (UFRA), under the identification number 7584300323.

Conflicts of Interest

The authors declare no conflicts of interest.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [ChatGPT and Grammarly] in order to [Improve the readability of the texts already formed]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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