

OVIPOSITION LAG IN NAZCA BOOBIES *SULA GRANTI*

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ABSTRACT

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Egg formation in well-studied poultry includes rapid yolk deposition (RYD), which completes the formation of the ovum, then ovulation and uptake of the ovum by the oviduct to complete egg formation. Roughly a day passes between the end of RYD and oviposition in these species. In other species, notably seabirds, egg formation is paused at the end of RYD for days or even more than a week. The phylogenetic coverage of data on this “oviposition lag” is patchy. We present the first estimate of oviposition lag for the Sulidae (4 d) and only the second estimate for the Suliformes.

Key words: vitellogenesis, Galápagos Islands, ovum retention

INTRODUCTION

Rapid yolk deposition (RYD; Grau 1984) begins the assembly of eggs from oocytes by female birds. Over a species-specific period lasting days to weeks (Réhault-Godbert & Guyot 2018), yolk material is added in a concentric fashion to the periphery of the ovum. After RYD is complete, the fully formed ovum transfers from the ovary to the oviduct during ovulation. Fertilization by sperm and the remainder of egg assembly (albumen, shell membranes, and mineral shell) occurs over approximately 24 h following ovulation (Gilbert 1971). If the end of RYD precedes ovulation by minutes or hours, as is usually the case with domestic poultry (Warren & Conrad 1939, Gilbert 1970), the “oviposition lag” from the end of RYD to laying is roughly 24 h.

In contrast to chickens, some seabirds have an oviposition lag of as much as 10 d, while the oviposition lag in other seabirds is as short as 2 d (Birkhead & del Nevo 1987, Astheimer & Grau 1990). The existence and duration of this lag has implications for the temporal pattern of energetic costs during reproduction (Astheimer & Grau 1990), for sperm competition and when an ovum can be fertilized (Birkhead & Montgomerie 2020, Hemmings & Birkhead 2020), and for the evolution of oviparity vs. viviparity (Anderson *et al.* 1987). In 1990, Astheimer & Grau summarized the current knowledge about the oviposition lag in seabirds (see also Birkhead & del Nevo 1987), showing that most information for birds, in general, came from seabirds in the Procellariiformes, Charadriiformes, and Sphenisciformes orders. Only a single result was available from the Suliformes (European Shag *Gulosus aristotelis*; Astheimer & Grau 1990), despite the order’s large number of taxa. To our knowledge, no additional results have been published for seabirds since 1990.

Here we provide the first data on oviposition lag in the Sulidae, from Nazca Boobies *Sula granti*. We measured the duration of the lag using scheduled feeding of lipophilic dyes to detect when RYD ended in relation to the time of egg laying.

METHODS

This study was conducted in the breeding colony of Nazca Boobies at Punta Cevallos, Isla Española, Galápagos Islands,

Ecuador (1°23’S, 89°37’W). This species breeds seasonally at Punta Cevallos, and most eggs are laid from October to January each year (Anderson 1993). Only one offspring is raised per breeding attempt, but two-egg clutches are common, providing an insurance egg to counter low hatching success and early nestling death (Clifford & Anderson 2001a, Humphries *et al.* 2006). During the 1996/97 and 1997/98 breeding seasons, we fed female Nazca Boobies fish (wild-caught Flathead Grey Mullet *Mugil cephalus* or sausages made from canned Pacific Sardine *Sardinops sagax*) containing a size 0 gelatin capsule filled with either red or blue lipophilic dye in a distinctive temporal sequence to show the timing of yolk formation and, by inference, when yolk deposition finished (Riddle 1909, Warren & Conrad 1939, Gilbert 1972) in relation to the date of egg laying. For example, Female 3 (Fig. 1) received six pills in the sequence blue (15 November), blue (20 November), red (24 November), blue (26 November), red (29 November), and red (03 December). Her egg was laid on 05 December; the yolk had four dyed rings in the sequence (center to periphery) blue-red-blue-red. Assuming that all pills administered during RYD are represented in the yolk, the last red pill produced no color in the yolk and must have been ingested after RYD was complete, represented as a “O” 2 d before oviposition in Fig. 1. Sudan IV dye (imparting the red color) was administered in two capsules, each containing 73 mg of dry dye powder, and Sudan Black (imparting the blue color) was administered in one capsule containing 153 mg of dry dye powder. We followed the methods of Clifford & Anderson (2001b), except that dye pills were provided to females every two days (longer in cases when females were away from their nest during experimental feeding or when they refused fish). We based our inference on the presence/absence of scheduled dye administrations in the yolk and not on counts of the full set of rings, avoiding the problematic assumption that yolk rings are laid down in a known daily schedule (Dobbs *et al.* 1976, Alisauskas & Ankney 1994).

During the 1996/97 breeding season we collected four B-eggs (the second-laid egg) on the day they were laid, and during the 1997/98 breeding season we collected eight B-eggs, also on the day they were laid, for a total of 12 eggs laid by females that had been fed dye pills. Collected eggs were boiled on the day of collection and sliced to compare the internal sequence of dye rings with the

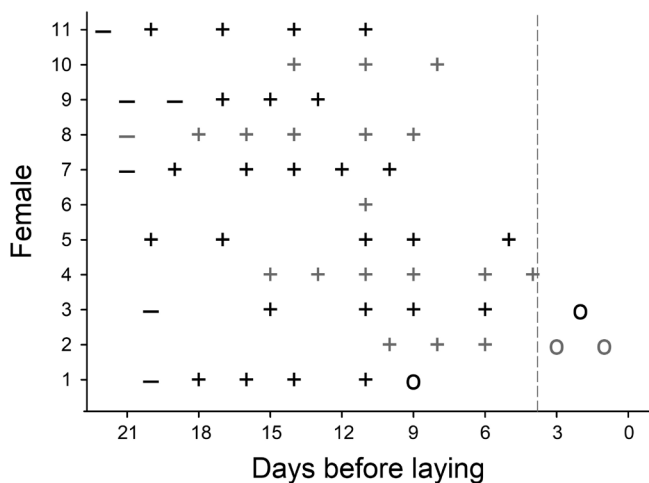


Fig. 1. Schedules of the appearance of lipophilic dyes in yolk rings in Nazca Booby *Sula granti* eggs. “-” indicates the time that a dye pill was administered but did not appear in the yolk before yolk formation began. “+” indicates a pill that did appear. “O” indicates failure to incorporate dye after a period of incorporation. Dashed line separates days on which dye was incorporated from days when dye was missing from yolks in two eggs and is adjacent to the time that dye incorporated weakly into the periphery of Female 4’s yolk. No rapid yolk deposition (RYD) occurred after 4 d preceding oviposition. Histories of different females are presented in different shades for visual clarity. Female 1’s O, 9 d before laying, was judged to be an error (see Discussion).

schedule of pill administration. Comprehensive daily nest histories were compiled for each female, providing the dates of egg laying. One female did not receive a dye pill during yolk formation and is not considered further. All birds in this study were part of a supplemental feeding study (Clifford & Anderson 2001b) in which they received supplemental fish as a vehicle for dye pills and variable amounts of additional supplemental fish.

This study was conducted under Wake Forest University Animal Care and Use Protocol A95-126.

RESULTS

Females 2 through 11 gave easily interpretable results (Fig. 1). Dye pills administered 18 to 4 d before the egg was detected always produced a discrete band of dye stain in the yolk. Pills given 3, 2, or 1 d before the egg was detected produced no staining.

Female 4 received a blue dye pill 4 d before the egg was detected, and its dye was weakly incorporated into the yolk, but only to the periphery; the surface of the boiled yolk was tinged blue, rather than a saturated yellow color. The weak staining in this case suggests that the dye arrived at the ovum in the last minutes of RYD and that less dye was incorporated than in interior, earlier, wider rings of dye staining. Given that lipophilic dye begins to be incorporated into yolk rapidly after ingestion (within 50 min in chickens; Gilbert 1970), Female 4’s blue pill was ingested during the afternoon, and no undyed yolk was overlaid on the dyed yolk, we can conclude that RYD in this yolk finished in the same afternoon that the dye was ingested. Female 4’s egg was absent in the morning 4 d later and

then present in the afternoon. Thus, RYD for this yolk was finished roughly 4 d before oviposition.

Female 1 produced a yolk with one dye ring, indicating that RYD ended roughly 10 d before laying, a finding that was dramatically inconsistent with data from the other females in the study (Fig. 1).

DISCUSSION

Nazca Boobies have a lag between the end of RYD and egg laying, as has been found in other seabird species (Birkhead & del Nevo 1987, Astheimer & Grau 1990). Most of our results are consistent with the interpretation that RYD ends approximately 4 d before oviposition. We consider Female 1’s contrary result unreliable. Female 1’s RYD began 19 d before the egg was detected (Fig. 1); if her RYD actually finished 10 d later, as Fig. 1 indicates, then that yolk would have been formed in slightly more than half the usual time (Clifford & Anderson 2001b). The rate of RYD in birds varies little within a species (Astheimer & Grau 1990), so a short period of RYD should produce a small yolk; however, Female 1’s yolk was not atypically small (pers. obs.), consistent instead with a failure to administer the last pill successfully. Thus, we conclude that the oviposition lag in Nazca Boobies is approximately 4 d.

The relationship of oviposition lag and the timing of ovulation within that lag is apparently unknown outside of poultry (Galliformes) and ducks (Anseriformes). Those taxa usually end RYD a few hours before ovulation, and infrequently as much as a day before ovulation (Warren & Conrad 1939, Gilbert 1970). We are unaware of other data bearing on the timing of ovulation in relation to oviposition in birds. Given this data gap, researchers generally assume that any oviposition lag longer than 24 h reflects time that the complete ovum spends in the ovarian follicle, after RYD is complete but before ovulation (Astheimer & Grau 1990, Réhault-Godbert & Guyot 2018, Hemmings & Birkhead 2020). Under this assumption, which is not contradicted with available data, some birds store a completed ovum in the ovary for days before the roughly 24-h period of ovulation, fertilization, and deposition of the rest of the egg.

Why this oviposition lag is apparently common in seabirds and not in other birds is unknown, but it may be connected to constraints associated with their foraging biology (Grau 1984, Hemmings & Birkhead 2020). Hypotheses for the causation of ovum storage (and oviposition lag) must address several obstacles. First, we do not know if the duration of the oviposition lag is under facultative control in seabirds; any degree of facultative control will obscure relationships among species and will require the sampling of large numbers of species to determine if relationships exist. Second, phylogenetic signal must be addressed appropriately, but presently, data only exist for 14 seabird species across four orders (Birkhead & del Nevo 1987, Astheimer & Grau 1990, this study), and these data show that phylogeny and foraging trip duration are confounded: the longest oviposition lags have been found in the order with the longest trips (Procellariiformes), and the shortest oviposition lags are concentrated in the Charadriiformes (most species in this order complete foraging trips within one daylight period). Larger samples in each seabird lineage will be required to separate phylogenetic from ecological effects.

Third, hypotheses for the cause of ovum storage and oviposition lag in seabirds must overcome the additional obstacle of accurately

measuring a female's uncertainty in colony arrival for egg laying with respect to ovulation; any such uncertainty would favor the capacity to store a completed ovum. For example, some taxa may have foraging trip lengths of a few hours regardless of conditions, with little uncertainty about when she can arrive at her nest, and therefore experience little selection to evolve an oviposition lag. However, selection pressure to evolve an oviposition lag may be stronger in other taxa that are subject to more complicated circumstances. In our study population of Nazca Boobies, foraging trips seldom exceeded one daylight period (Anderson & Ricklefs 1987) until 1997. Then, a multi-decadal change in diet in 1997 (Tompkins *et al.* 2017) led to longer trips at more distant sites: foraging trips typically lasted 2 d or more, and a significant proportion of trips lasted > 4 d (Zavalaga *et al.* 2011, Howard *et al.* 2021, McKee *et al.* 2023). How to interpret this variability in the context of past selection for an oviposition lag is not straightforward.

Linking oviposition lag to seabird foraging characteristics is tempting, but the limited data that are currently available do not yet provide robust support for this association. Aside from seabirds, galliform poultry, and ducks, we are aware of only one other estimate of oviposition lag in birds, from the Emu *Dromaius novaehollandiae*: its lag of 10 d (Hirsch & Grau 1981) rivals that of albatrosses (Astheimer & Grau 1990). Calder & Rowe (1977) used body weight increments, not yolk structure, of Southern Brown Kiwis *Apteryx australis* to infer a 14-d oviposition lag, a suggestive result that we view with caution until a more direct measure of RYD is used. The study of oviposition lags needs wider sampling than is presently available, both within seabirds and across bird species in general.

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REFERENCES

ALISAUSKAS, R.T. & ANKNEY, C.D. 1994. Costs and rates of egg formation in Ruddy Ducks. *The Condor* 96: 11–18.

ANDERSON, D.J. 1993. Masked Booby (*Sula dactylatra*). In: POOLE, A. & GILL, F. (Eds.) *The Birds of North America*, No. 73. Philadelphia, USA: The Academy of Natural Sciences.

ANDERSON, D.J. & RICKLEFS, R.E. 1987. Radio-tracking masked and blue-footed boobies in the Galápagos Islands. *National Geographic Research* 3: 152–163.

ANDERSON, D.J., STOYAN, N.C. & RICKLEFS, R.E. 1987. Why are there no viviparous birds? A comment. *American Naturalist* 130: 941–947.

ASTHEIMER, L.B. & GRAU, C.R. 1990. A comparison of yolk growth rates in seabird eggs. *Ibis* 132: 380–394.

BIRKHEAD, T.R. & DEL NEVO, A.J. 1987. Egg formation and the pre-laying period of the common guillemot *Uria aalge*. *Journal of Zoology* 211: 83–88.

BIRKHEAD, T.R. & MONTGOMERIE, R. 2020. Three decades of sperm competition in birds. *Philosophical Transactions of the Royal Society B* 375: 20200208.

CALDER, W.A. & ROWE, B. 1977. Body mass changes and energetics of the kiwi's egg cycle. *Notornis* 24: 129–135.

CLIFFORD, L.D. & ANDERSON, D.J. 2001a. Experimental demonstration of the insurance value of extra eggs in an obligately siblicidal seabird. *Behavioral Ecology* 12: 340–347.

CLIFFORD, L.D. & ANDERSON, D.J. 2001b. Food limitation explains most clutch size variation in the Nazca booby. *Journal of Animal Ecology* 70: 539–545.

DOBBS, J.C., GRAU, C.R., ROUDYBUSH, T. & WATHEN, J. 1976. Yolk ring structure of quail subjected to food deprivation and refeeding. *Poultry Science* 55: 2028–2029.

GILBERT, A.B. 1970. Yolk deposition in the chicken oocyte and its relationship with ovulation. *Journal of Reproduction and Fertility* 23: 539–540.

GILBERT, A.B. 1971. Transport of the egg through the oviduct and oviposition. In: BELL, D.J. & FREEMAN, B.M. (Eds.) *Physiology and Biochemistry of the Domestic Fowl, Volume 3*. New York, USA: Academic Press.

GILBERT, A.B. 1972. The activity of the ovary in relation to egg productions. In: FREEMAN, P.M. & LAKE, P.E. (Eds.) *Egg Formation and Production*. Edinburgh, UK: British Poultry Science, Ltd.

GRAU, C.R. 1984. Egg formation. In: WHITTOW, G.C. & RAHN, H. (Eds.) *Seabird Energetics*. New York, USA: Plenum Press.

HEMMINGS, N. & BIRKHEAD, T.R. 2020. Extraordinary sperm to egg ratios in seabirds. *Marine Ornithology* 137: 1–8.

HIRSCH, K.V. & GRAU, C.R. 1981. Yolk formation and oviposition in captive Emus. *The Condor* 83: 381–382.

HOWARD, J.L., TOMPKINS, E.M. & ANDERSON, D.J. 2021. Effects of age, sex, and ENSO phase on foraging and flight performance in Nazca boobies. *Ecology and Evolution* 11: 4084–4100. doi:10.1002/ece3.7308

HUMPHRIES, C.A., AREVALO, V.D., FISCHER, K.N. & ANDERSON, D.J. 2006. Contributions of marginal offspring to reproductive success of Nazca booby (*Sula granti*) parents; tests of multiple hypotheses. *Oecologia* 147: 379–390.

MCKEE, J.L., TOMPKINS, E.M., ESTELA, F.E. & ANDERSON, D.J. 2023. Age effects on Nazca booby foraging performance are largely constant across variation in the marine environment: Results from a 5-year study in Galápagos. *Ecology and Evolution* 13: e10138. doi:10.1002/ece3.10138

RÉHAULT-GODBERT, S. & GUYOT, N. 2018. Vitellogenesis and yolk proteins, birds. *Encyclopedia of Reproduction* 6: 278–284. doi:10.1016/B978-0-12-809633-8.20568-2

RIDDLE, O. 1909. The rate of growth of the egg-yolk in the chick, and the significance of white and yellow yolk in the ova of vertebrates. *Science* 27: 245.

TOMPKINS, E.M., TOWNSEND, H.M. & ANDERSON, D.J. 2017. Decadal-scale variation in diet forecasts persistently poor breeding under ocean warming in a tropical seabird. *PLoS One* 12: e0182545. doi:10.1371/journal.pone.0182545

WARREN, D.C. & CONRAD, R.M. 1939. Growth of the hen's ovum. *Journal of Agricultural Research* 58: 875–893.

ZAVALAGA, C.B., EMSLIE, S.D., ESTELA, F.A., MÜLLER, M.S., DELL'OMO, G. & ANDERSON, D.J. 2011. Overnight foraging trips by Nazca boobies and the risk of attack by predatory fish. *Ibis* 154: 61–73.