



RESEARCH ARTICLE

Data loggers in artificial eggs reveal that egg-turning behavior varies on multiple ecological scales in seabirds

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ABSTRACT

In most avian species, egg-turning behavior during incubation is vital for proper embryonic development and hatching success. However, changes in turning behaviors are rarely studied across different temporal scales (e.g., day–night or across incubation phases), though the timing of incubation behaviors affects reproductive success. We used data loggers encapsulated in artificial eggs to measure turning rates and angle changes of eggs in Western Gull (*Larus occidentalis*) and Laysan Albatross (*Phoebastria immutabilis*) nests. We examined diurnal and daily cycles in egg-turning behaviors across early, middle, and late incubation phases. Our results indicate that (1) egg-turning behaviors remain similar throughout incubation, resulting in a consistent environment for developing chicks; (2) egg-turning rates and angle changes vary according to diurnal cycles and day length in each species; and (3) egg-turning rates, but not angle changes, were similar between species. Egg-turning behaviors may vary among species according to seasonality and geography, and using consistent methodologies to measure egg turning will further clarify the role of egg turning in avian life history and ecology.

Keywords: biologging, egg rotation, egg turning, incubation, Laysan Albatross, parental care, Western Gull

Des enregistreurs de données dans des œufs artificiels révèlent que le comportement de retournement des œufs varie à des échelles écologiques multiples chez les oiseaux de mer

RÉSUMÉ

Chez la plupart des espèces aviennes, le comportement de retournement des œufs au cours de l'incubation est vital pour assurer le bon développement embryonnaire et le succès d'éclosion. Cependant, les changements dans les comportements de retournement des œufs sont rarement étudiés à différentes échelles temporelles (p. ex., jour/nuit ou phases d'incubation), bien que le moment où se produisent les comportements de couvain affecte le succès reproducteur. Nous avons utilisé des enregistreurs de données encapsulés dans des œufs artificiels afin de mesurer les taux de retournement et les changements d'angle des œufs dans les nids de *Larus occidentalis* et de *Phoebastria immutabilis*. Nous avons examiné les cycles diurnes et quotidiens dans les comportements de retournement des œufs au cours des phases de début, de milieu et de fin d'incubation. Les résultats indiquent que : 1) les comportements de retournement des œufs demeurent similaires tout au long de l'incubation, ce qui résulte en un environnement constant pour les oisillons en développement; 2) les taux de retournement des œufs et les changements d'angle varient en fonction des cycles diurnes et de la durée du jour pour chaque espèce; et 3) les taux de retournement des œufs, mais pas les changements d'angle, étaient similaires entre les espèces. Les comportements de retournement des œufs peuvent varier entre les espèces selon la saisonnalité et la géographie; l'utilisation de méthodologies cohérentes pour mesurer le retournement des œufs clarifiera davantage le rôle du retournement des œufs dans le cycle vital et l'écologie des oiseaux.

Mots-clés: biologging, rotation des œufs, retournement des œufs, incubation, *Phoebastria immutabilis*, soins parentaux, *Larus occidentalis*

INTRODUCTION

Parental care behaviors affect offspring phenotype and reproductive fitness (Clutton-Brock 1991). In most bird

species, adults must incubate eggs to ensure proper embryonic development and reproductive success. Avian incubation behaviors can be mediated by the physical and biotic environments. For instance, birds may alter

incubation behavior in response to weather patterns (Thierry et al. 2013b), predation risk (Basso and Richner 2015), and ambient temperature (McClintock et al. 2014). Certain parameters of avian incubation—such as egg temperature, nest humidity and water vapor conductance, and parental attendance—shift during the course of incubation, creating variable microclimates for the developing embryo over time (Ar and Rahn 1980, 1985, Cooper and Voss 2013, DuRant et al. 2013, Portugal et al. 2014). Adults may respond to age-related changes in these parameters by altering their incubation behaviors. For example, increased egg-cooling rates over the course of incubation resulted in shorter, but more frequent, incubation recess bouts in Black-capped Chickadees (*Poecile atricapillus*) to keep eggs at an optimal temperature while maintaining the energetic requirements of the incubating adult (Cooper and Voss 2013). Thus, the timing of adult behaviors in concert with the physiological needs of the embryo, the parent, and environmental patterns and processes can influence avian breeding success (Lack 1968, Wang and Beissinger 2009).

Another incubation behavior, egg turning, has rarely been examined in wild birds, although it is vital to embryonic development and hatching success (New 1957, Tullett and Deeming 1987). Periodic movement of the egg eliminates diffusion gradients in the albumen, aiding sub-embryonic fluid formation and the utilization of albumen nutrients via vascularization by the embryo (Deeming 1989). Unlike most reptile and megapode eggs, failure to turn avian eggs decreases surface area of the avian-specific chorioallantoic membrane, impeding embryonic gas exchange and albumen absorption involved in sub-embryonic fluid formation (Tazawa 1980, Deeming 1991). As a result, the embryos of unturned eggs have decreased oxygen consumption and lowered heart rates (Pearson et al. 1996), longer incubation times (Tullett and Deeming 1987), lower-than-normal mass (Tullett and Deeming 1987), and significantly reduced hatching success compared with turned eggs. There are also temporal constraints on egg turning. Eggs of domestic fowl have greatest hatching success when turned throughout the first third of incubation and also during 3–5 days before pipping (New 1957, Tona et al. 2005). The needs of avian embryos differ as the embryo ages, but no studies have described egg-turning changes across the incubation period in wild birds (Deeming 2002, Cooper and Voss 2013). Deeming (2002) showed strong relationships between egg-turning rate and 2 factors that covary: percent albumen content and hatchling precociality. Generally, more altricial species have greater albumen content in the egg (Ricklefs 1977, Sotherland and Rahn 1986), which correlates with increased egg-turning rate (Deeming 2002). This pattern was significant across a wide range of avian

species, which suggests that phylogeny and developmental mode influence egg-turning rates.

Comparatively less is known about the importance of egg turning angles in avian incubation. Turning angles associated with egg turns influence egg viability; an increased turning angle can improve egg hatchability (Van Schalkwyk et al. 2000) and an angle of $>35^\circ \text{ turn}^{-1}$ reduces the incidence of embryo malposition, even in the event of fewer turns per hour (Elibol and Brake 2006a). However, controlled studies of egg turning in incubators do not turn eggs $>180^\circ$ on a single axis (Van Schalkwyk et al. 2000, Elibol and Brake 2006a)—though, presumably, eggs could be turned through 360° on a single axis. Like egg-turning rates, there is large variation in the degree of angle change within avian species, between 1° and $180^\circ \text{ turn}^{-1}$ (Graul 1975, Shaffer et al. 2014). However, variation in angle changes among avian species is not well described or attributed to other life-history traits of birds. Reports of changes in mean angle per turn during the incubation period are mixed—Mallards (*Anas platyrhynchos*) lessened the mean degree of angle change through incubation (Caldwell and Cornwell 1975), but Mountain Plovers (*Charadrius montanus*) did not (Graul 1975). More data on egg turning angle, and its relationship to turning rate, are needed to assess the importance of the degree of angle change during avian incubation.

Numerous researchers have attempted to evaluate egg turning in wild birds using visual observations (e.g., Beer 1965, Drent 1970, Caldwell and Cornwell 1975) or remote logging devices (e.g., Beaulieu et al. 2010, Thierry et al. 2013a, 2013b), where egg-turning rates are characterized in turns per hour per day or for the entirety of incubation. Although informative, these methods were unable to capture movement of a three-dimensional object or record egg-turning behavior at high resolution ($\sim 1 \text{ Hz}$) and thus reported turning rates and angle changes on 1 or 2 axes (Shaffer et al. 2014). The lack of a comprehensive and standardized method for measuring egg-turning behavior in wild birds may have hindered examination of relationships between egg-turning behavior and environmental processes. As such, the influence of ecological variables on egg-turning behavior, such as diurnality or the length of day and night, is rare in the literature, though turning rates have fluctuated with time of day and/or parental turnover in captive waterfowl eggs and with diurnality in Cassin's Auklets (*Ptychoramphus aleuticus*), but not in Western Gulls (*Larus occidentalis*) or Laysan Albatrosses (*Phoebastria immutabilis*) (Howey et al. 1984, Gee et al. 1995, Shaffer et al. 2014). Given known relationships between incubation behaviors and the physical environment, recording the egg-turning behaviors of wild birds should enable deeper investigation into possible relationships between environment and egg-turning behavior.

TABLE 1. Life-history and incubation parameters of the two study species. Early, middle, and late incubation phases were defined by the authors.

	Laysan Albatross	Western Gull
Clutch size	1	3
Egg mass ^a	261 g	81 g
Mean albumen content ^a	63.9% ^b	66.8% ^b
Nest turnover rate	2–4 wk ^c	Every 2–4 hr ^d or daily
Incubation period	November–February	April–June
Length of incubation	63–65 days	30 days
Early	0–19 days	0–9 days
Middle	20–39 days	10–19 days
Late	40+ days	20+ days
Hatchling precociality ^a	Semi-precocial	Semi-precocial
Adult body mass	2,520–3,040 g ^e	800–1,250 g ^d
Diel patterns	Diurnal	Diurnal

^aData are from Deeming (2007).

^bPercentage reflects mean albumen content by order (Procellariiformes vs. Charadriiformes).

^cTickell (2000).

^dPierotti and Annett (1995).

^eKappes et al. (2010).

Using the same microtechnology as Shaffer et al. (2014), we assessed egg-turning rates and angle changes in Western Gulls and Laysan Albatrosses. We were interested in egg-turning rates and changes (1) in diurnality, because both species are active in the daytime; and (2) across the incubation period, due to the importance of the timing of egg-turning behaviors in controlled studies. Because these species are similar in egg albumen content, hatchling maturity (semi-precocial), and diurnality, we expected egg-turning rates and activity patterns to be similar between these 2 species (Table 1). We also expected both species to turn eggs more often during the first third of incubation (hereafter “early incubation”) compared with later periods. These analyses examine the conclusions of lab-based avian incubation studies in wild contexts, thus linking critical questions surrounding avian evolution, development, and behavior.

METHODS

Study Species and Sites

We studied breeding Western Gulls (hereafter “gulls”) at Año Nuevo Island Reserve, California, USA (37.1083°N, 122.3371°W), and Laysan Albatrosses (hereafter “albatrosses”) at Kaena Point Natural Area Reserve, Oahu, Hawaii, USA (21.5749°N, 158.2784°W). These species exhibit some similarities and differences in incubation parameters and life-history patterns (Table 1) that interact with their behavioral patterns, including egg attendance.

Artificial Egg Design

Artificial egg prototypes were created from 3.175 mm thick vacuum-formed white polystyrene plastic at San José State University. Subsequently, in November 2013, eggs for fieldwork were made on a 3D printer. Eggs were made in 2 halves that were held together by an interlocking mechanism or threads. In addition to the data logger (see below), we added modeling clay and wire-pulling lubricant to each artificial egg to approximate the mean mass of real eggs for each species without interfering with logger function (Table 1). We also painted gull eggs with nontoxic, acrylic paint to mimic the coloration of real gull eggs (see Shaffer et al. 2014: fig. S1). Details on egg design are described more fully in Shaffer et al. (2014).

Logger Specifications

Data loggers consisted of a triaxial accelerometer and magnetometer, a temperature thermistor, a microcontroller that logged measurements once every second, and a lithium battery that powered the loggers for up to 8 days. The accelerometer registered egg-orientation changes in roll, pitch, and yaw (*x*-, *y*-, and *z*-axis) attitudes, and magnetometer measurements adjusted attitude changes for magnetic north.

Logger Deployments

Pilot tests of logger performance inside artificial eggs were conducted using a Top Hatch Incubator (Brower Equipment, Houghton, Iowa, USA) as described in Shaffer et al. (2014). Egg logger deployments in gull nests occurred in May–June 2013 (*n* = 32) for an average of 4.30 ± 1.95 days (Table 2). We excluded data from 5 loggers that recorded data for <24 hr (Table 2). To minimize observer bias, blinded methods were used when all behavioral data were recorded and/or organized.

Because there are hundreds of gull nests on Año Nuevo Island, we selected study nests on the basis of nest accessibility while minimizing disturbance to parents and neighboring nests in the colony. Although gulls lay multi-egg clutches of variable size, modal clutch size is 3 eggs. To control for variations in clutch size, all study nests were either (1) increased in clutch size from 2 to 3 eggs by adding an artificial egg containing an egg logger; or (2) maintained at a clutch size of 3 by removing 1 viable gull egg to a surrogate nest in the colony and replacing it with an artificial egg for the duration of the deployment period. We tracked study and surrogate nests weekly to (1) track any changes in clutch size and hatching success, (2) replace egg loggers in artificial eggs, and (3) remove artificial eggs and return viable eggs to their original nest before chicks hatched.

We chose study nests on the basis of egg viability. About 30% of albatross pairs at Kaena Point are female–female pairs, which likely results in a relatively high rate of

TABLE 2. Deployment and analysis metadata. "Year" indicates the year that each dataset was started, because the Laysan Albatross breeding season extends into 2 calendar years. Days analyzed are means \pm SD.

Species	Year	Total deployments (<i>n</i>)	Deployments analyzed (<i>n</i>)	Nests analyzed (<i>n</i>)	Days analyzed per deployment (<i>n</i>)	Hatching success (%)
Laysan Albatross	2012	20	14	5	6.11 \pm 1.77	NA ^a
Laysan Albatross	2013	40	32	10	4.79 \pm 2.25	NA ^a
Western Gull	2013	32	27	18	4.30 \pm 1.95	91.7 ^b

^a Because sampled Laysan Albatross eggs were infertile, hatching success could not be analyzed.
^b Indicates percentage of hatched eggs in experimental and surrogate nests combined.

nonviable eggs (Young et al. 2008). The single egg in each albatross nest was candled 10–14 days after laying. If the egg was infertile (i.e. clear albumen with no blood vessels), it was removed, collected for contaminant sampling, and replaced with an artificial egg containing an egg logger. Artificial eggs remained in nests at Kaena Point and were checked weekly to replace the egg logger with cleared memory cards and recharged batteries. After the deployment period was completed, each artificial egg was removed, leaving the nest empty and allowing study albatrosses to end the year's breeding attempt, which would have failed anyway.

We deployed egg loggers in albatross nests in December 2012–January 2013 ($n = 20$, with repeated deployments in 5 nests) and December 2013–January 2014 ($n = 40$, with repeated deployments in 10 nests) for an average of 6.11 ± 1.77 days in 2012–2013 and 4.79 ± 2.25 days in 2013–2014 (Table 2). In 2012, four of the nests in which we deployed loggers were abandoned, and 2 abandonments occurred because the artificial egg opened during deployment. The printed eggs used in 2013 resulted in no abandonments. We excluded data from 2 loggers in 2012 and 8 loggers in 2013 that recorded for <24 hr (Table 2).

Logger Processing

Raw logger data were processed using custom routines in MATLAB release 2012a (Mathworks, Natick, Massachusetts, USA), where 3-2-1 Euler angles were translated from accelerometer and magnetometer data and used to determine egg orientation and angle changes. We then applied Euler's rotation theorem to define the minimum *total* angular change required to go between egg orientations at successive time steps, as fully described in Shaffer et al. (2014). After applying a smoothing function to reduce sensor noise, a threshold of 0.03 rad s^{-1} was applied to initial data. Any data that exceeded this threshold indicated a rotation event. However, to filter minute and indirect egg movements by the adult, only rotations that logged a minimum total angular change of $>10^\circ$ required to move between egg orientations were defined as rotation events. Thus, each rotation event logged a turn as well as the minimum total angular change of that turn. The first 2 hr of each deployment were

excluded from analysis, as well as the last 2 hr if the logger was still recording during retrieval. Any deployments that included abandonment or were not recorded for a full 24 hr were also removed from the study.

Incubation phase was determined by conducting weekly nest checks and following the hatching success (gulls only) or nest checks every 1–3 days after laying (albatrosses) of each experimental nest, assuming incubation periods of 30 days for gulls and 63–65 days for albatrosses (Table 1). If we could not accurately determine lay date, we excluded the nest from analysis. Early, middle, and late incubation phases were determined by dividing the entire incubation period into thirds (Table 1) to mirror controlled studies on incubation phase of fowl (New 1957, Elibol and Brake 2006b).

Times of sunrise and sunset and total day or night length were determined using ephemeris tables based on geographic location of each colony and calendar dates of deployments. Given the time of year that each species breeds, gulls experienced increasing day lengths across the incubation period, whereas albatrosses experienced decreasing day lengths until winter solstice (December 21) and increasing day lengths afterward. However, given the tropical latitude of Kaena Point, day length varied little (<1 hr) for albatrosses during our study. Ephemeris tables indicated that gulls experienced average day lengths of 14 hr, whereas albatrosses experienced day lengths of 11 hr.

The total numbers of turns per nest per 24 hr period were distilled into mean hourly turning rates for each hour of deployment by nest. Hourly rates were averaged within individual nests by 24 hr period, daytime, and nighttime periods in each 24 hr period to obtain a mean hourly turn rate in each nest per period. Mean angle change per turn of the egg was obtained by summing the minimum total angular change per rotation event within individual nests per 24 hr period, daytime period, and nighttime period of each deployment and dividing by the number of turns observed in each period, resulting in a mean angle change per turn in each nest per period. Subsequent averaged data were filtered into early, middle, and late incubation phases per period.

Statistical Tests

We compared the mean hourly turn rate per day and the mean angle change per turn in 24 hr periods and day–

night temporal periods throughout incubation phases. Some deployments spanned 2 incubation phases and varying totals of 24 hr periods, daytime, and nighttime data among nests, creating variations in sample size among incubation phases and nests. Therefore, we performed a linear mixed model on all 24 hr period data. The mixed effects models used either hourly turn rate per day or mean angle change turn per day as a response variable, with incubation phase and species as fixed effects and nest number as a random effect. We were also interested in quantifying the mean daily movement of gull eggs and albatross eggs. First, we calculated the mean egg-turning rate per day and mean angle change turn per day for each nest ($n = 10$ for gulls, $n = 15$ for albatrosses). We multiplied each of these means within nests to obtain a mean total angular change (movement) per egg per day. This value was multiplied by the length of incubation for each species (30 days for gulls, and 63 days for albatrosses) to obtain the mean total movement of each egg for the entire incubation period. We compared daily movement data and total movement data, separately, between gulls and albatrosses using Welch's 2-sample t -tests.

We also performed a series of tests to determine whether egg-turning behavior varied diurnally. We were interested in (1) trends within the daytime and nighttime data separately and (2) comparisons of the daytime and nighttime data. To address the first question, we performed mixed linear models to determine whether daytime or nighttime turning rate and angle changes differed across the incubation period for both species. Model parameters matched the model parameters for 24 hr period data within species. Secondly, we wanted to compare daytime data to nighttime data to determine whether birds engaged in different egg-turning behavior in nighttime vs. daytime. Thus, we performed a paired-sample t -test (or Wilcoxon signed-rank test, reported as z values, where data were not normally distributed) between daytime and nighttime data.

We determined significance for all statistical tests using an alpha of 0.05, and tests of normality and heteroscedasticity were performed on all data. We calculated effect size for paired-sample t -tests using Cohen's $d = |M| / SD$, where M is the mean of differences and SD is the standard deviation of differences. Pairwise comparison tests were performed where appropriate, but the results are not reported because the findings were insignificant. Statistical tests were performed in R (R Development Core Team 2015). Values are reported as means \pm SE unless otherwise stated.

RESULTS

Daily Data

Linear mixed models indicated that turning rates were not significantly different between species ($\chi^2 = 0.05$, $P = 0.82$)

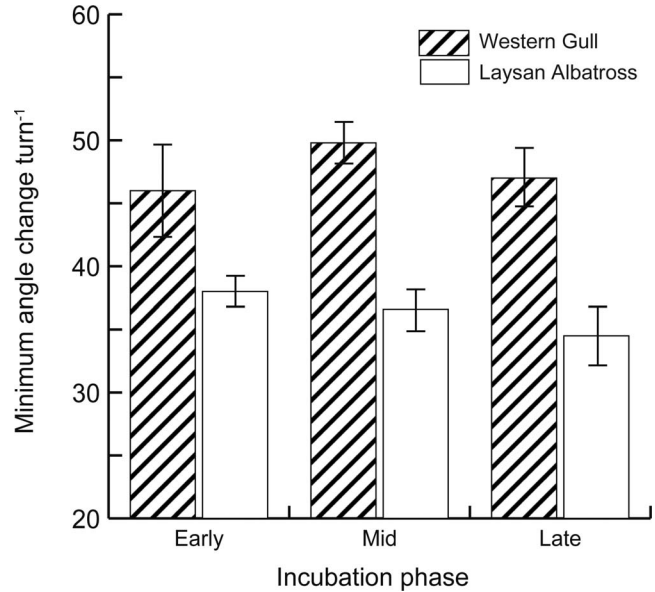


FIGURE 1. Minimum angle change per turn (mean \pm SE; expressed in degrees) for Western Gulls and Laysan Albatrosses in 24 hr periods across time during incubation at our study sites. Angle change differences between species were significant within all incubation phases within this time scale.

or among incubation phases ($\chi^2 = 0.24$, $P = 0.62$). Although incubation phase did not have an effect on angle change between gulls and albatrosses ($\chi^2 = 1.97$, $P = 0.16$), the effect of species was significant for angle changes ($\chi^2 = 19.31$, $P < 0.001$), mean angle changes for gulls being $11.4 \pm 2.2^\circ$ greater than mean angle changes for albatrosses (Figure 1). Given the similarity in turning rates but differences in angle changes, total daily movement for gull eggs ($2,892 \pm 166^\circ$) was significantly greater ($t_{18} = -3.16$, $P = 0.006$) than that for albatross eggs ($2,249 \pm 118^\circ$) by $\sim 640^\circ$. However, mean total egg movement over the incubation period differed ($t_{22} = 6.15$, $P < 0.001$) by $\sim 55,000^\circ$ between gulls ($86,769 \pm 4,981^\circ$) and albatrosses ($141,698 \pm 7,406^\circ$), which is a function of the disparate length of the incubation period between these species.

For gulls, turning rates ($F_{1,23} = 0.55$, $P = 0.47$) and angle changes ($F_{1,23} = 0.04$, $P = 0.84$) did not significantly vary among early, middle, and late incubation phases. Similarly, turning rates ($F_{1,33} = 0.54$, $P = 0.47$) and angle changes ($F_{1,33} = 2.12$, $P = 0.16$) did not vary significantly among incubation phases for albatrosses.

Comparisons within Daytime and Nighttime Data

Neither species ($\chi^2 = 0.32$, $P = 0.57$) nor incubation phase ($\chi^2 = 2.20$, $P = 0.14$) significantly affected turning rates between species during daytime. Similarly, nighttime turning rates did not differ between species ($\chi^2 = 0.82$, $P = 0.36$) or incubation period ($\chi^2 = 2.20$, $P = 0.14$), which suggests that regardless of diurnal period or incubation

phase, gulls and albatrosses turn their eggs at similar rates (Figure 2A, 2C).

There was a species-level effect on daytime angle changes ($\chi^2 = 7.75$, $P = 0.01$), whereby daytime angle changes in gulls were $7.8 \pm 2.7^\circ$ greater than daytime angle changes in albatrosses. Incubation phase did not significantly influence angle changes ($\chi^2 = 0.95$, $P = 0.33$). However, there was a significant interaction between species and incubation phase whereby nighttime angle changes were greater in gulls than in albatrosses by $20.2 \pm 2.1^\circ$ ($\chi^2 = 40.7$, $P < 0.001$) and nighttime angle changes decreased by $3.2 \pm 0.7^\circ$ from early to late incubation ($\chi^2 = 13.80$, $P < 0.001$; Figure 2B, 2D). These results indicate that diurnal angle changes are different between species, but shifts in angle changes across the incubation period are either not significant (daytime) or only subtle (nighttime).

Comparisons of Daytime Data to Nighttime Data

Gulls turned their eggs more often during the day than at night. This pattern was significant during the early ($t_6 = 4.46$, $P = 0.004$, $d = 1.67$) and late ($t_6 = 4.26$, $P = 0.005$, $d = 1.64$) incubation phases, and nearly so during middle incubation ($z = -1.89$, $P = 0.06$). Turning rates were ~ 1 turn hr^{-1} more frequent during the day (3.2 ± 0.3) than at night (2.2 ± 0.1 ; Figure 2A) during early incubation, and about half a turn per hour more frequent in the day (2.6 ± 0.2) than at night (2.1 ± 0.2) during late incubation.

Gulls moved their eggs over a larger angle at night than during the day. This pattern was significant during the early ($t_6 = -5.77$, $P = 0.001$, $d = 2.18$) and late ($t_6 = -2.63$, $P = 0.04$, $d = 0.99$) incubation phases, and the trend was similar in middle incubation ($t_9 = -1.46$, $P = 0.18$). The magnitude of angle changes was $\sim 15^\circ$ less during the day ($41.6 \pm 3.7^\circ$) than at night ($56.5 \pm 2.5^\circ$) during early incubation but only $\sim 7^\circ$ less during the day ($45.3 \pm 3.4^\circ$) than at night ($52.0 \pm 2.4^\circ$) during late incubation.

In albatrosses, daily patterns of turning rates differed during early ($t_{14} = 5.21$, $P < 0.001$, $d = 1.35$) and middle ($t_{14} = 3.54$, $P = 0.003$, $d = 0.92$) incubation phases, but not during late incubation ($z = -0.67$, $P = 0.50$). Albatrosses turned eggs about one turn per hour more during the day (2.9 ± 0.2) than at night (1.9 ± 0.2) in early incubation, and about half a turn per hour more during the day (3.0 ± 0.2) than at night (2.5 ± 0.2) in middle incubation.

In contrast to gulls, the difference in magnitudes of angle changes between day and night was less distinct in albatrosses (Figure 2D). For albatrosses, angle changes between daytime and nighttime periods differed only during middle incubation ($t_{14} = 2.94$, $P = 0.01$, $d = 5.04$), in which eggs were turned $\sim 3^\circ$ more during the day ($37.2 \pm 1.7^\circ$) than at night ($34.4 \pm 1.3^\circ$). The magnitude of angle changes was not significantly different between day and night during early ($t_{14} = 1.08$, $P = 0.30$) or late ($z = -1.75$, $P = 0.08$) incubation phases.

DISCUSSION

Previous research has shown that interspecific comparisons of egg-turning rates and angle changes are highly variable (e.g., Howey et al. 1984, Deeming 2002), which prompted controlled studies of optimal egg-turning rates and angle changes to maximize hatching success in laboratory conditions (e.g., Robertson 1961, Tona et al. 2005, Elibol and Brake 2006a). Recent discussions have focused on changes in egg-turning rates of wild birds due to other variables, such as human disturbance (Beaulieu et al. 2010), hormone fluctuations (Thierry et al. 2013a, 2013b), weather conditions (Thierry et al. 2013b), and nest turnover patterns (Shaffer et al. 2014). However, their analyses presumed that egg-turning rates and angles remained temporally static throughout incubation, though studies have suggested that turning of avian eggs is most vital during early and late incubation (New 1957, Tona et al. 2005, Deeming 2009). Indeed, changes in egg temperature through time indicate that incubation conditions reflect the behavior of the nesting adult (Cooper and Voss 2013, DuRant et al. 2013) or diurnal patterns (Shaffer et al. 2014). Our results demonstrate that egg-turning behaviors in Western Gull and Laysan Albatross are indeed diurnally dynamic but do not vary during phases of the incubation period.

Egg-turning Behaviors Are Similar Across the Incubation Period

Neither species exhibited variation in egg-turning behaviors during different incubation phases. This finding was surprising, given previous research on the importance of egg turning during early incubation (New 1957, Tona et al. 2005, Deeming 2009). Unlike other incubation behaviors that are adjusted across the incubation period, gulls and albatrosses may not need to adjust egg-turning behavior. This may be because the benefits of turning an egg during early incubation outweigh any negative effects of turning an egg at similar angles throughout incubation. In the context of this study, observed temporal similarities in albatross egg-turning behaviors may be due to the infrequent turnover rate of incubating albatrosses. Because albatross incubation shifts can last beyond 4 wk, repeated deployments in each nest may have logged the activities of a single bird for several days or even weeks (Tickell 2000). However, this explanation cannot be applied to gulls, which exchange incubation duties every 4–24 hr (Annett and Pierotti 1999, C. A. Clatterbuck et al. personal observation). Because both species exhibit biparental incubation strategies, it may be important for both sexes to engage in similar egg-turning behaviors. Differences in egg-turning behavior between the sexes may be more pronounced in species that exhibit egg neglect or uniparental incubation, though more data are needed to

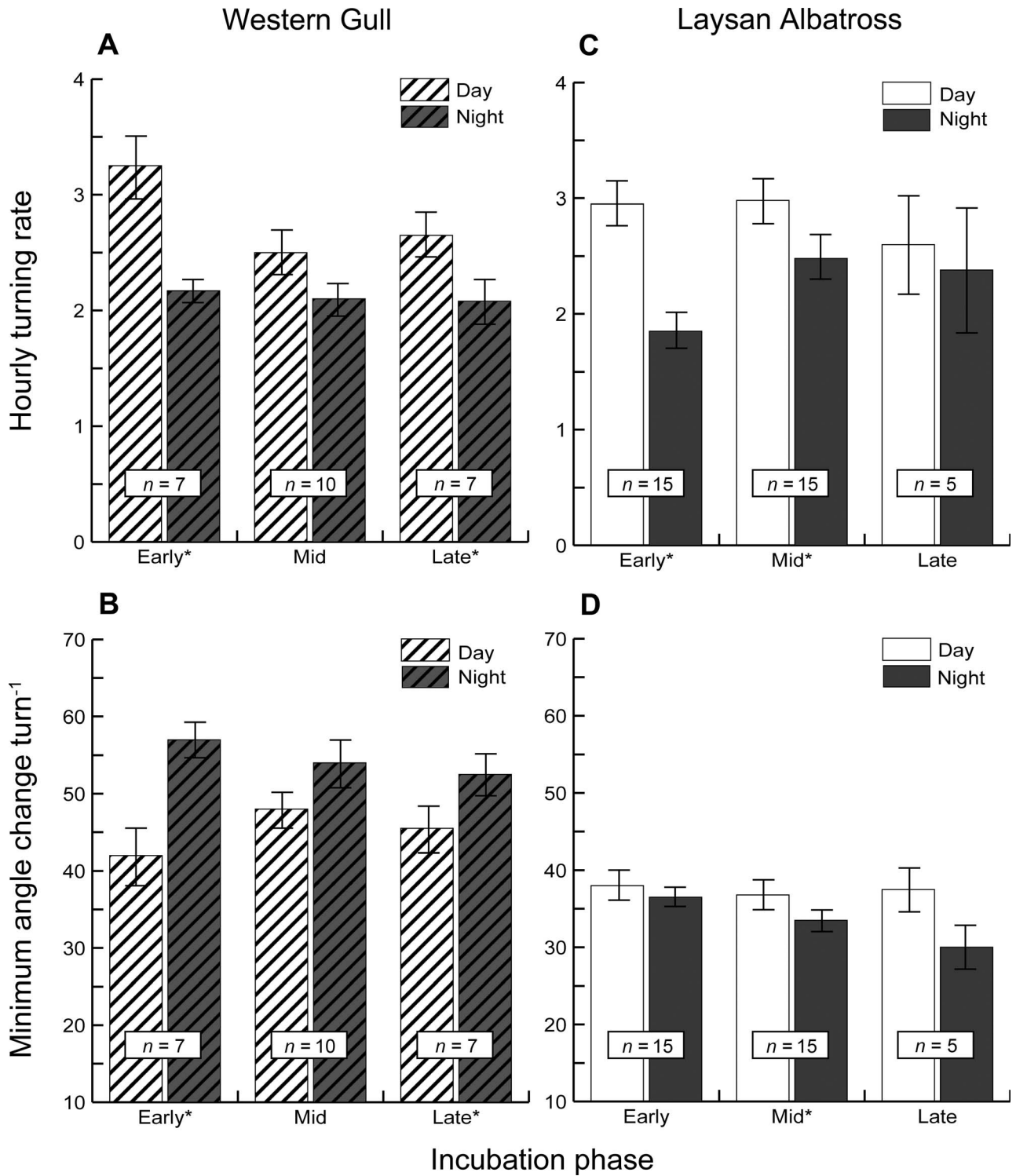


FIGURE 2. Comparisons of diurnal turning rates and angle changes (mean \pm SE) across incubation phases for (A, B) Western Gulls and (C, D) Laysan Albatrosses (n = number of nests sampled) at our study sites. Asterisks represent significant differences between day and night within the appropriate incubation phase.

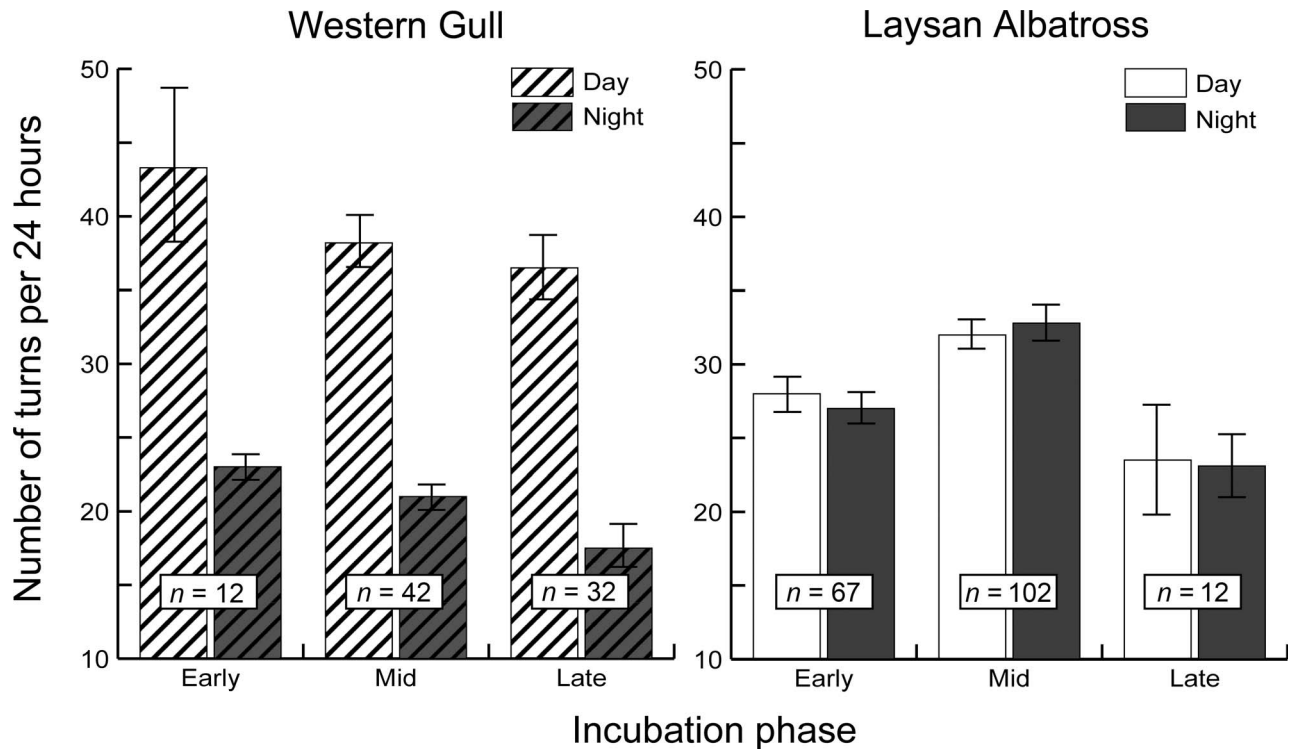


FIGURE 3. Number of turns (mean \pm SE) in a 24 hr period across incubation phases for Western Gulls and Laysan Albatrosses at our study sites (n = number of 24 hr periods sampled). Only 24 hr periods that recorded an entire day or entire night were sampled. Mean daytime and nighttime rates from entire dates were multiplied by the number of hours in daytime or nighttime and then averaged by the number of dates sampled for each species.

support this claim (Reneerkins et al. 2011, DuRant et al. 2012, Cooper and Voss 2013).

Diurnal Variation in Egg-turning Behaviors

The diurnality of gulls and albatrosses influenced turning rate in similar ways, with more egg turns during the day than at night, as is true for waterfowl and cranes (Howey et al. 1984, Gee et al. 1995). In gulls, daytime and nighttime patterns in egg-turning rates and angle changes differed during early and late incubation phases. In general, gulls turned eggs more often in daytime but at smaller angle changes, whereas at night they turned eggs with less frequency but at greater angle changes (Figure 2A, 2B). Though gulls are generally quiescent at night, these data indicate that parents continued to actively turn their eggs. Further, the gull colony is dense at Año Nuevo Island, and territorial adults often engaged in aggressive behaviors against conspecifics during the breeding season, much like other gull species (Pierotti and Annett 1994). Thus, it is possible that greater daytime turning rates were caused by colony and adult activity, whereas the relative lack of activity on the colony at night allowed incubating adults to rest. However, adults may increase the turning angle at night to compensate for the lack of activity and, thus, fewer turns. In this manner, parent gulls can achieve similar

overall egg turning with fewer turns at night. To our knowledge, the present study is the first to show temporal associations between turning rate and angle change in wild birds. Why daytime and nighttime egg-turning patterns were similar during middle incubation is unclear, but it may be indicative of fewer differences between daytime and nighttime adult activity during this incubation phase.

Albatrosses also exhibited diurnality in egg-turning rate, but less variation in angle changes over day and night cycles than gulls. For albatrosses, turning rates in daytime were greater during early and middle incubation than during nighttime, but angle changes only differed slightly during middle incubation (Figure 2C, 2D). Albatrosses also follow diurnal cycles when present in the colony, but the density of albatross nests at Kaena Point was lower than at most albatross colonies (Arata et al. 2009, L. C. Young personal observation). Thus, albatrosses at Kaena Point are less likely to be influenced by colony activities and intraspecific interactions than typical albatross colonies across the North Pacific. At the latitude of Kaena Point, combined with the time of year (December–January), the length of nighttime was an hour longer than daytime, resulting in approximately equivalent numbers of turns in day and night periods (Figure 3). Thus, although albatross parents turned their eggs more often during the day, a

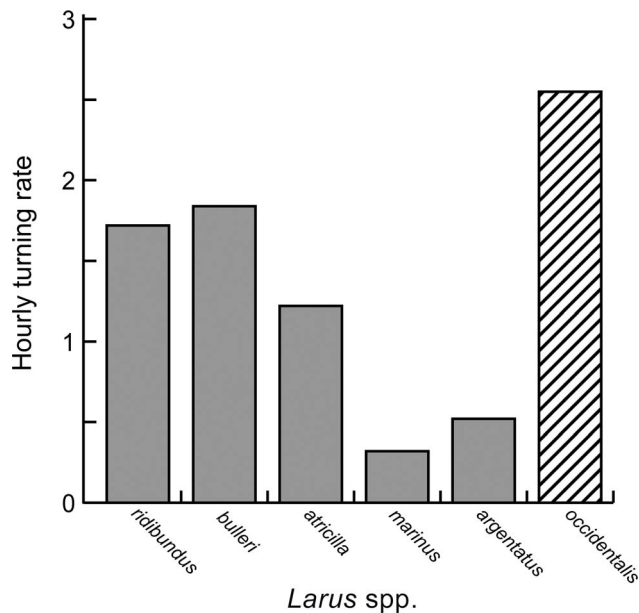


FIGURE 4. Mean hourly turning rates for species in the genus *Larus*. All data except those for *Larus occidentalis* (present study) are compiled from Beer (1961; *L. ridibundus*), Beer (1965; *L. bulleri*), Drent (1970; *L. argentatus*), Impekoven (1973; *L. atricilla*), and Butler and Janes-Butler (1983; *L. marinus*) as reported in Deeming (2002: table 11.1). All data except those for *L. occidentalis* were collected via visual observations of incubating adults at the nest. Data are sorted by phylogenetic relationships from Pons et al. (2005), in which *L. marinus* and *L. argentatus* were more closely related to *L. occidentalis* than the other 3 species. *Larus marinus* and *L. argentatus* also have similar incubation periods (29–30 days) and egg mass (93–116 g) to *L. occidentalis* (Table 1; Deeming 2002).

longer nighttime period compensates for a lower nighttime turning rate. The similar length of day and night may explain why egg turning angles were also relatively similar throughout incubation phases and the diurnal cycle for albatrosses.

Variability in Egg-turning Behaviors Between Species

Angle changes, but not turning rate, differed daily between species (Figure 1), but these differences were more profound when analyzed by diurnal cycle (Figure 2C, 2D). Why might angle changes, but not turning rates, vary between species? Beyond the length of day and night, as discussed previously, one possibility is that egg turning angle is a function of egg size, mass, and method of turning. For instance, it is likely more difficult to rotate a larger egg than a smaller egg, resulting in smaller angle changes through time. Additionally, incubating birds may have more control over how often to turn an egg than the magnitude of the angle change, especially because egg turns appeared to take place with the feet and body and not the bill in these species. However, gulls appeared to have some control in egg turning angle between day and

night (Figure 2C). Additionally, egg size is proportional to adult body mass in both species studied here (Tables 1 and 2). Weight asymmetry due to embryonic growth also may affect angle change, though this was not examined in our study (Deeming 2002).

Though turning rate and angle changes did not vary by incubation period alone, when separated by diurnal cycle, egg-turning behaviors emerged that were not obvious in Shaffer et al. (2014). Differences between early, middle, and late incubation may be more obvious in species that exhibit uniparental incubation or egg neglect, like many passerines, shorebirds, waterbirds, and some burrowing seabirds (Reneerkens et al. 2011, DuRant et al. 2012, Cooper and Voss 2013). Examining incubation behaviors on multiple time scales (diurnal and across incubation phase) may be necessary to capture the subtle but dynamic features of avian incubation behaviors. Future studies should consider both the behavior and life history of their subject species and examine behaviors based on these time scales.

Microtechnology Use in Assessing Incubation Behaviors

A variety of observational methods have been used to record egg-turning behavior, and findings suggest a nearly linear relationship between egg albumen content, hatching precocity, and egg-turning rate (Deeming 2002, 2009). However, when turning rates of Western Gulls were compared with reported mean egg-turning rates for other gull species, turning rate is dissimilar among species in the genus *Larus*, which are similar in egg albumen content and hatching precocity (Figure 4). The variation in egg turning among *Larus* species may be due to how egg turning was measured (*sensu* Shaffer et al. 2014), which has included a mixture of methods (e.g., observations, marked eggs, or remote technology). This poses a challenge for scientists who wish to put the results of egg-turning studies using sensitive technology in context with current theories that compare incubation behavior to life history and avian phylogeny.

Interestingly, mean angle changes appear to be more variable between species in the present study than has previously been reported. While birds exhibit a wide range of angle changes when turning, variation among species from a variety of orders range from 52° to 90° (reviewed in Deeming 2002), whereas gulls and albatrosses in the present study exhibit smaller mean angle changes, which may be due to egg mass (Table 1 and Figure 1). Variation in angle changes in our study compared to other studies may be due to the manner in which angle changes were recorded. The loggers we used had triaxial sensors, whereas other studies used less sensitive sensors (e.g., Howey et al. 1984, Gee et al. 1995, Beaulieu et al. 2010) with greater margins of error (e.g., $\pm 22.5^\circ$ in Gee et al.

1995 vs. $\pm 2\text{--}4^\circ$ in each axis of the loggers in this study). As microtechnology becomes more affordable and readily available, it may be easier to examine the relationships between egg-turning rates, angle changes, and numerous other variables known to affect incubation among avian species at varying temporal and spatial scales.

Future Studies

Many variables affect adult birds during incubation, including short-term weather disturbances (Thierry et al. 2013b), climatic conditions (Deeming 2002), field metabolic rates (Shaffer et al. 2001), and human disturbance (deVilliers et al. 2006, Beaulieu et al. 2010). Optimal hatching success relies on adult behavior, so evaluating the interaction of a multitude of ecological factors with egg-turning behavior over time will further define relationships between egg turning, developmental mode, and avian ecology. Continued study of egg-turning patterns through time would be particularly enlightening for species that exhibit different incubation patterns than the gulls and albatrosses studied here, especially when combined with other incubation parameters, like incubation temperature (Cooper and Voss 2013, Kelsey et al. 2016). Lastly, combining methodological techniques to assess egg turning, such as cameras and remote egg loggers, may clarify links between avian incubation and adult behaviors at the nest.

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Ethics statement: Animal research was conducted as approved by the San José State Institutional Animal Care and Use Committee (SJSU no. 980), California Department of Fish and Wildlife (SC-11994), Migratory Bird Treaty Act (MB74834N-0), U.S. Geological Survey Bird Banding Laboratory, California Parks, University of California Natural Reserve System, Hawaii Division of Forestry and Wildlife and Natural Area Reserve System, and U.S. Fish and Wildlife Service. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions: C.A.C. and S.A.S. formulated research questions and design. C.A.C., L.C.Y., E.A.V., and S.A.S. performed the experiments and wrote the paper. A.D.N., G.C.B., and S.A.S. developed the egg logger designs and data processing scripts. C.A.C. and S.A.S. analyzed the data.

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