SUPERNORMAL DELAY IN HATCHING, EMBRYO COLD TOLERANCE AND EGG FOSTERING IN THE BLACK GUILLEMOT *CEPPHUS GRYLLE*

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SUMMARY

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We observed an apparent suspension of embryonic growth in the Black Guillemot *Cepphus grylle*, a cavity-nesting alcid typically having a two-egg clutch and incubation period of ≈ 28 d. Limited suspension of embryonic growth is common for species with a multi-egg clutch, but our observations are apparently the first to document an extended (≥14 d) suspension prior to the onset of incubation in the wild. We found no previous record of the fostering and successful incubation of the egg of the previous owners by birds usurping a nest cavity. Observations were made on Cooper Island, in northern Alaska, where man-made structures on a sand and gravel substrate provide nest cavities. At the time of clutch completion a previously non-breeding pair displaced the original nest owners and pushed the two eggs to the edge of the nest depression. Approximately 7 d later the usurping pair moved the displaced eggs back into the nest depression. They added their own egg to the clutch 14 d later and began incubating all three eggs. The initial egg in the original clutch pipped after 28 d of incubation (45 d after deposition), but died while pipping. The other hatched after 33 d of incubation (47 d after deposition); indicating embryonic growth had been suspended for ≥ 14 days. During the period of growth suspension, daily temperatures averaged 2.9°C (range 0.1-7.4°C) with daily minima < 0°C on 12 of the 18 days, demonstrating an extended cold tolerance in the species.

Key words: *Cepphus grylle*, incubation, cold-tolerance, egg-fostering, Arctic

INTRODUCTION

The avian embryo can experience a period of suspended growth after egg deposition and before the onset of incubation (Feast *et al.* 1998). This suspension of embryonic growth requires temperatures too low to allow development and high enough to avoid embryo death (White & Kinney 1974). It also requires humidity sufficient to limit evaporative water loss but low enough to minimize microbial growth on eggshells (Cook *et al.* 2005). For species with a multi-egg clutch, this postponement of development allows an increase in hatching synchrony when incubation is withheld until clutch completion (Hébert 2002). In avian husbandry and poultry science, the storage of viable eggs prior to incubation has practical applications, where Chicken *Gallus gallus* (Funk & Forward 1960), Turkey *Melagris gallopavo* (Bakst & Gupta 1997) and Ostrich *Struthio camelus* (Hassan *et al.* 2005) eggs can be stored ≤14 d before the onset of incubation.

While the temporal and thermal tolerances of the preincubated embryos of domesticated species have been well studied, there is little information for wild species (Arnold *et al.* 1987, Stoleson & Beissinger 1999) with most information on egg neglect in the wild obtained after incubation has begun (Boersma & Wheelwright 1979, Gaston & Powell 1989, Sockman & Schwabl 1998, Webb 1987). We report here on the apparent suspension (≥ 14 d) of development in eggs of the Black Guillemot *Cepphus grylle*, a cavity-nesting seabird, following the intraspecific displacement of the parents from the nesting cavity immediately after egg-laying. After the extended period of suspension at extremely low ambient temperatures (<5°C), the displacing foster parents incubated the eggs with their own for a normal 28-d incubation period resulting in one of the eggs hatching and the other pipping.

The circumstances leading to a period of extended preincubation neglect followed by normal incubation would be rare in nature, as would the environmental conditions allowing survival of the embryo during growth suspension. While postponement of incubation and embryonic growth is common for species with a multi-egg clutch, the observations presented here are apparently the first to document an extended (≥14 d) suspension of embryonic growth prior to the onset of incubation in the wild. The low ambient air temperatures at our arctic study colony provide evidence of an extended cold tolerance by the embryo of an arctic seabird. Additionally, there appears to be no previous record of the fostering and successful incubation of the egg of the previous owners by birds usurping a nest cavity.

METHODS

Black Guillemot breeding chronology and success were studied in 2005 as part of a long-term study on Cooper Island, Alaska (71°14'N, 155°42'W), 30 km east southeast of Point Barrow. The species nests in cavities, typically in rock crevices (Storer 1952), but all nests on Cooper Island are in artificial boxes or wooden debris sitting on a sand and gravel substrate (Divoky *et al.* 1974). The majority of the breeding adults on the island have been banded with unique combinations of color bands, allowing identification of nest-site occupants. We determined the dates of laying and hatching for the 141 active nests on the island in 2005 by examining all nest cavities daily during the presumed period of egg-laying (15 June to 10 July). Eggs were measured to the nearest 01.mm (length and width) with calipers and labeled according to laying order on the first nest-check after laying when a parent bird was not on the nest. Once a clutch was initiated we examined the nest cavity daily until the clutch had two eggs, the maximum clutch size for a single female, or for the following six days, the longest observed intra-clutch interval. To limit disturbance to incubating parents we made infrequent checks of a nest cavity until 25 d after clutch initiation. After 25 d, a nest was examined daily until all eggs hatched or were determined to be inviable. Chicks were weighed daily to the nearest 1 g with a spring scale. Temperature data were obtained from the hourly observations taken by the National Weather Service in Barrow, 35 km west of the colony.

Observations reported here were from a nest site that consisted of a 1 m x 0.5 m piece of plywood propped lengthwise on its edge by a 10 cm high plank. A piece of wood abutting one end of the plywood limited the cavity to a single entrance. The resulting nest cavity was 90 cm deep with a slanted ceiling 12 cm at its highest point. The closed end of the nest cavity had a nest depression in the sand and gravel approximately 15 cm in diameter and 5 cm deep.

RESULTS

The pair that occupied the nest site at the start of the 2005 breeding season was displaced during the period of egg-laying. The birds were present at the site on our arrival on 15 June and had bred there annually since 1995—a male banded as an adult in 1987 and at least 19 years old in 2005, and a 14-year-old female that fledged from Cooper Island and first bred in 1995. They were regularly seen together at the nest site until 21 June, but the male was not seen again in 2005 or 2006. The disappearance of a breeding bird from the colony during the breeding season is typically indicative of mortality, based on a lack of resightings in subsequent breeding seasons (G. Divoky unpubl. data). On 24 June a single fresh egg was found in the nest cavity approximately 5 cm from the nest depression. A second egg was found on 27 June also 5 cm from the nest depression and 15 cm away from the first egg. The eggs are assumed to be from the same female, as the difference in volume index (length x breadth2) was only 4% (colony mean = 3.5%, SD = 2.55, n = 111) and the intra-clutch laying interval was 3 d (mean = 2.86 d, SD = 1.06, n = 96), both similar to intra-clutch variation observed at other purported single female clutches in 2005. The long-term female owner, who is assumed to have laid the first two eggs, was last recorded as occupying the nest site on 27 June and after that date was seen only as part of the nonbreeding population within 30 m of her previous nest site, but not associated with a nest cavity or mate.

On 28 June, the day after the second egg was laid, the nest site was occupied by an unbanded pair of guillemots that were actively entering the site and defending it from nonbreeding prospectors and the previous female owner. Intra-specific displacement of eggs by nonbreeding guillemots occurs at a small number (<5%) of nests every year (G. Divoky, unpubl. data) and it is likely that the pair occupying the site on 28 June displaced the two eggs at the site. The displacement of the eggs would have been facilitated by the absence of the long-term owner male, because the male performs the majority of nest-site defense during egg-laying (Nelson 1987, G. Divoky unpubl. data). The two eggs from the previous owners were still in the nest cavity and out of the nest depression on 28 and 30 June and 1 July. On 1 July, one member of the new pair was in the cavity but not incubating the eggs. By 4 July both eggs had been moved into the nest depression and one member of the new pair was in the nest site on that date and also on 8 and 10 July. On 11 July, 14 d after the two-egg clutch was completed; a third egg was added to the nest (Fig. 1). Its volume index was 19% smaller than the average of the first two eggs, indicating it was probably not produced by the same female. The 14 d interval between the occupation of the site by the replacement pair and the appearance of the third egg indicates it was likely produced by the replacement female. Access to a nest cavity has been shown to be an important proximate stimulus to ovulation in cavity-nesting alcids (Sealy 1975) and egg formation for Black Guillemots takes approximately 14 d (Petersen 1981). After the third egg had been added to the clutch, nest checks on 20, 23 July and 1, 4, 5 and 7 August found all three eggs being incubated by the replacement pair.

On 8 August, 45 d after deposition, the first egg in the clutch was pipping (a single 5 mm hole) and contained a viable chick which by 13 August had died while still in the eggshell. A chick of 30 g hatched from the second egg on 13 August, 47 d after laying, and was 35 g on 14 August. Mean hatching weight for the colony was 33.1 g (n = 136 SD = 2.32). Brooding parents prevented nest examination on 15 August and the chick was dead at 40 g on 16 August. The third egg, laid by the usurping pair, did not hatch and showed no sign of development when examined on 20 August after the nest was abandoned.

DISCUSSION

We believe the two eggs laid by the original owners experienced a period of suspended embryonic growth that was caused by a lack of incubation from deposition (24 and 27 June, respectively) to the time the foster parents added a third egg to the clutch (11 July). This was followed by a normal development period when the eggs were incubated by the foster parents. The original owners could have provided little or no incubation before displacement as the male disappeared before clutch initiation and the female's time at the colony would be limited during the period of egg formation and deposition. In addition, the original location of the eggs, out of the depression, not adjacent to each other and with one in a location that

Fig. 1. Supernormal Black Guillemot clutch produced through displacement of original site owners and incubated by displacing pair. The single egg laid by the displacing pair is the one on the right with the visible "1" (photo: Nora Rojek).

would not accommodate an incubating adult, indicates that little incubation could have occurred before the eggs were moved to the nest depression on 2-4 July. The period out of the nest depression lasted 8-10 d for the first egg and 5-7 d for the second egg. This was followed by 7-10 d in the nest depression for both eggs prior to the deposition of the third egg. The eggs were apparently moved to the nest depression by the new owners and at least one member of that pair was seen in the site directly adjacent to the nest depression in the period before the appearance of their own egg, so some incubation could have occurred during the 7-10 d. The duration and effectiveness of any incubation attempted would be expected to be less than optimal as the new female would have reduced colony attendance during the period she was producing an egg. Also brood patch development might not be complete for either parent until near the appearance of their own egg.

The apparent suspension of embryonic growth in the two original eggs is demonstrated by their pipping and hatching after a near-normal period of incubation beginning with the appearance of the third egg. The first egg pipped 28 d after the third egg was added to the clutch, indicating that had it survived it would have hatched at 29-30 d of incubation. The second egg hatched after 33 d of incubation. Both incubation periods are slightly longer than the mean interval between egg deposition and hatching in other nests in 2005 (mean = 27.8 d, range 25-33 d) but indicate that little embryonic growth occurred before the third egg was added to the clutch. Assessing the effects the suspension of growth had on subsequent development of the embryo and the quality of hatchlings is confounded by the eggs being incubated as part of a three-egg clutch. Three-egg clutches occur in guillemots when a second female lays an egg in an occupied nest (Cairns 1981, G. Divoky unpubl. data) and are regular but uncommon in the species (Petersen 1981) comprising 2% of the 600 nesting attempts on Cooper Island in 2002-2005 (G. Divoky unpubl. data). *Cepphus* have a bi-lobed brood patch (Storer 1952) that does not completely cover three eggs resulting in incomplete incubation in supernormal clutches. Three-egg clutches have lower hatching success and longer incubation periods than one- and two-egg clutches (Petersen 1981), having only 15% hatching success (n = 11 nests) at our study colony from 2002-2005, compared to the 75% in one- and two-egg clutches over the same period.

The two eggs experienced extremely low temperatures during the period of growth suspension, though avian embryos tolerate extreme cold better than they tolerate extreme heat (Batt & Cornwell 1979). The mean daily air temperature during the 18 d between the laying of the first egg and presumed start of incubation (24 June – 11 July) was just above freezing at 2.9°C (range 0.1-7.4°C) with daily temperature minima of < 0°C on 12 of the 18 days. While solar radiation can temporarily increase nest cavity temperatures by 2-3°C in the afternoon (G. Divoky & B. Harter unpubl. data), temperatures in the nest cavity were almost certainly <5°C for most of the time. While the periodic occupation of the nest cavity by the displacing pair could have increased the temperatures the eggs experienced before incubation, the survival of the embryos under the conditions described is noteworthy. Growth of the avian embryo is suspended at ambient temperatures below physiological zero which is approximately 24-27°C (White & Kinney 1974, Wilson 1991) with growth occurring at a range of 36-38°C (Drent 1973; Webb 1987). Domestic chicken eggs are kept at 15°C when stored for subsequent incubation (Bakst & Gupta 1997, Tona *et al.* 2003). Lower temperatures appear to be better for long-term suspension of growth as there is an inverse relationship between optimum storage temperatures and length of storage for chicken eggs (Funk & Forward 1960, Kirk *et al.* 1980). A thermal environment warmer than physiological zero but below normal incubation temperature causes asymmetric development of embryonic tissues that, when the suboptimal temperatures persist, can result in abnormal development and mortality from unsynchronized growth (Deeming & Ferguson 1992, Romanoff & Romanoff 1972).

Seabird eggs, especially those of cavity nesters, are thought to have increased tolerance to hypothermia associated with egg neglect (an interruption of incubation of a day or more) necessitated by trophic and predator avoidance constraints that can disrupt incubation schedules (Gaston & Powell 1989). Egg neglect with retained viability can last as long as 7 d in offshore feeders such as the Fork-tailed Storm-Petrel *Oceanodroma furcata* (Boersma & Wheelwright 1979), but would be expected to be uncommon in the nearshore feeding Black Guillemot where neglect should be less frequent and of shorter duration. However, guillemot embryos, especially those in cold environments, could be expected to have a high tolerance for neglect before the initiation of incubation, because guillemots lay a two-egg clutch with a 2-6 d intra-clutch interval, pre-incubation chilling of the first egg is common. Also, the individuals breeding at our study colony may have increased cold tolerance as they are part of a population that persisted in an unglaciated section of the Arctic Basin (Kidd & Friesen 1998) during the last glaciation and have occupied the region since. The daily average temperature for the region during the last two weeks of June, when most clutches are initiated and initial eggs would be unattended, is 2.9°C (range 1.7-3.9°C). The nest-sites on Cooper Island are snow-covered until just before egg-laying with nest substrate frozen in some nests at the time of egg deposition (Divoky 1998). Selection for embryonic cold tolerance could be expected to be greater for this population than for the more subarctic or temperate populations of the species (Nettleship & Evans 1985).

Unincubated eggs are vulnerable to extremes of humidity that can increase evaporative water loss or the potential for microbial infections (Beissinger *et al.* 2005). The ambient relative humidity during the period of presumed growth suspension was 80% (NWS), similar to the 75% humidity recommended for storing poultry eggs (Tona *et al.* 2003). The risk of microbial infection of eggshells, that can reduce hatching success in humid tropical habitats (Cook *et al.* 2003), is probably low for Black Guillemots in the Arctic because of the relatively low humidity, protection from precipitation offered by the nest site and the near freezing temperatures.

There are apparently no previous reports of a pair of birds usurping a nest and then successfully fostering the eggs of the previous site owners. The adoption of the eggs is all the more surprising since the usurping pair displaced them during the takeover of the nest site and then returned them to the depression after they had secured ownership. This would appear to have negative consequences for the usurping pair, even when the fostered egg did not hatch, as it would typically result in a supernormal clutch with resulting decreased incubation efficiency and hatching success.

While the circumstances reported here are exceptional, our observations demonstrate that extended suspension of embryonic growth can occur in the wild and at near freezing temperatures. The circumstances where a 14 d suspension of growth would be adaptive would rarely occur in the wild. The supernormal delay we observed would appear to be an extension of the more adaptive short-term suspended embryonic development that occurs during the intraclutch laying interval, which permits a delay in initiation of incubation when required.

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