Hatching Vocalisations in Free-Living Rockhopper Penguins

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Hatching vocalisations in free-living Rockhopper Penguins

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We analysed the vocalisation behaviour of Rockhopper Penguin chicks *Eudyptes chrysocome* during hatching. Hatching vocalisation rates (in number per minute) were not consistently influenced by ambient air temperature. They did not differ between chicks hatching from first-laid eggs (which are smaller, hatch second, and usually suffer brood reduction) and chicks hatching from second-laid eggs (which usually develop into fledglings). Hatchlings did not show significantly different vocalisation rates in the presence of a sibling during hatching. Hatching vocalisation rates also did not increase between the penultimate day and the last day before hatching. These results suggest that Rockhopper Penguin chicks might gain little benefit (parental care) from increased vocal behaviour at the late incubation stage.

Key words: Rockhopper Penguin, hatching vocalisation behaviour, egg dimorphism, air temperature
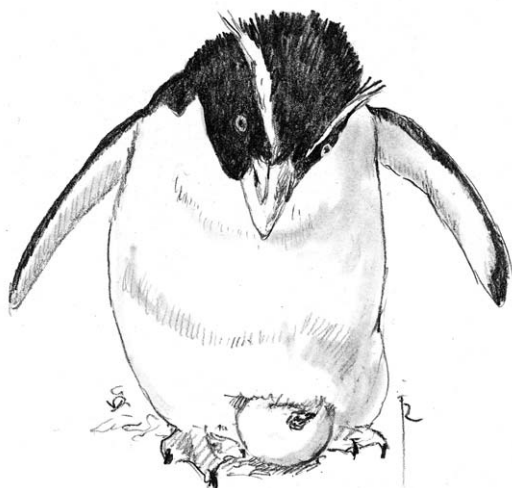
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Nestlings indicate their need for food, heat or protection with a complex begging display, generally involving gaping, posturing and vocalisation behaviours (Wright & Leonard 2002). Parents use this information to adjust their investment in parental care for a single nestling or between competing siblings (Godfray & Johnstone 2000, Wright & Leonard 2002, Grodzinski & Lotem 2007).

Hatchlings also produce vocalisations while they are still in the egg (Oppenheim 1972, Evans 1988). These vocalisations can be heard several days before hatching in precocial birds and during hatching in altricial birds (Gottlieb & Vandenbergh 1968, Oppenheim 1972). Their rate usually increases as hatching approaches (see Bugden & Evans 1999 on domestic chicken *Gallus gallus*) and is almost continuous at the late pipped egg stage for some birds (Evans 1988). Laboratory studies have shown that hatchlings also produce more vocalisations when exposed to temperatures above or below mean incubation values at the pipped egg stage (Evans 1990, Bugden & Evans 1999) or shortly before this stage (Brua *et al.* 1996).

These vocalisations might be the first signal to parents that hatchlings are viable and will soon hatch (Brua *et al.* 1996). Moreover, as almost all avian hatchlings are ectothermic (Vleck & Vleck 1996), this calling behaviour could also affect parents' incubating behaviour. Bird parents are indeed known to increase their incubating behaviour when vocalisation rates increase (Impekoven 1973, Evans 1988, 1992, Brua *et al.* 1996).

While hatching vocalisations can be costly, either energetically (Abraham & Evans 1999) or in terms of predation (see Haskell 1994, Leech & Leonard 1997 for begging behaviour), they might constitute an honest signal of hatchling needs for parental care.

Crested penguins (genus *Eudyptes*) lay a fixed number of two eggs, on average four days apart. The second-laid egg (commonly called the B-egg) is always larger than the first-laid egg (A-egg) and usually hatches one to three days before the A-egg (Warham 1975, Williams 1995). Hatching time (from pipping to hatching) does not differ significantly between A- and B-eggs in Rockhopper Penguins *E. chrysocome* (mean ± SD: 35.0 ± 10.2 hours, $n = 103$, Poisbleau and Demongin, unpubl. data). In crested penguins, due to potential differences in parental care during incubation, hatching and brooding, B-eggs and chicks are generally less exposed to predation, better insulated and less likely to be actively or passively ejected by parents or first-hatched chicks than A-eggs. Brood reduction therefore almost always occurs in favour of B-eggs and chicks (Gwynn 1953, Warham 1975, Lamey 1990). This consistent within-clutch variation makes crested penguins relevant models for the study of hatching vocalisation behaviour in siblings issued from eggs with very different characteristics, and for the testing of hypotheses about its potential role in brood reduction.

In this study, we analysed the hatching vocalisation behaviour of Rockhopper Penguins to investigate (1) whether hatching vocalisations are a means to solicit parental care by analysing the effect of temperature on vocalisation rates, (2) if this behaviour differs between A- and B-chicks and (3) whether the presence of a sibling affects vocalisation rates. Other environmental variables that could influence vocalisation rates (i.e. wind or precipitation) were controlled by the experimental design (hatching eggs were placed in a box during recording). Additionally, we (4) examined whether hatchlings signal their imminent readiness to hatch, by testing for an increase in vocalisation rates between the penultimate day and the last day before hatching.

Study site and birds

The study was carried out at the "Settlement colony" on New Island, Falkland Islands (51°43'S, 61°17'W) during two breeding seasons (from October to December in 2006 and 2007). The colony has around 5000 pairs of Rockhopper Penguins breeding in an open or semi-open (interspersed with and fringed by Tussac Grass *Poa flabellata*) habitat. After the arrival of the first males (early October), we visited the colony

daily to mark active nests and subsequently to monitor the eggs, all marked with unique codes using a non-toxic marker. We selected 100 nests in 2006 and 158 nests in 2007. In some nests, we removed one of the two eggs on the day after the B-egg was laid in order to obtain an obligate A- or B-egg clutch ($n = 24$ A-nests and 22 B-nests in 2006 and 20 A-nests in 2007) for the purposes of other studies (see Poisbleau *et al.* 2008, 2009a, 2009b).

Hatching vocalisation in Rockhopper Penguins consists of a succession of almost identical phrases and the variability of individuals' vocalisations is very low compared to other bird species (Jouventin 1982). The vocalisation structure of this species is much simpler than in other penguin species (Jouventin 1982). Moreover, sonograms of Rockhopper Penguin chick calls are quite amorphous (Jouventin 1982). We therefore do not expect the acoustic structure of vocalisations to play an important role in hatchling–parent communication, compared to vocalisation rates in this species. Therefore, we chose to investigate hatching vocalisation rates (the importance of these calls for the communication between hatchlings and parents has been explained in the introduction) rather than other measures in this first study investigating hatchling vocalisations in penguins.

Methods

During the hatching period, we made daily inspections of each egg close to its hatching date. Incubating parents did not move from the nest during egg manipulation and continued to incubate empty nests during recordings. Each hatching egg was individually collected and immediately placed in a cylindrical box (see Rubolini *et al.* 2005 for details) equipped with a microphone (Sony ECM-CS10 Professional Microphone) and connected to a digital recorder (Sony Digital Recorder ICD-P520). The interval between removal from the nest and the start of the recording was always less than 30 sec, and thus very similar among eggs. During this transport time, the egg was kept warm in our hands. The same settings were used for all recordings: the recording sound volume was retained at maximum and a microphone was placed 1 cm opposite the eggshell hole. We recorded vocalisations only from eggs with eggshell holes of around 1 cm in diameter (beak visible). For comparison, typical egg volumes (mean ± SD) are 83.5 ± 9.4 cm^3 for A-eggs and 106.8 ± 8.5 cm^3 for B-eggs (Poisbleau *et al.* 2011). Eggs just cracked or with a bigger hole were not recorded. We thus recorded all the hatching chicks at the same developmental stage, ensuring the validity of comparisons. When an

egg that we had already recorded met the criteria again the following day, the first recording was attributed to the penultimate day before hatching, and a second recording was made.

We recorded vocalisations for 14 minutes, during which the boxes were left in a protected area within the Tussac Grass five metres away from the colony. In these conditions, boxes were protected from the wind and the eggs were all in the same environmental conditions, with a sound contact with the colony. Following Rubolini *et al.* (2005), we excluded the first two minutes of each recording from acoustical analyses to allow the hatchlings to resume normal activity after manipulation.

Air temperature was measured by an automatic weather station close to the colony. Although absolute temperatures here were probably colder than at the colony, we assume that these data give a good relative assessment of ambient conditions in the colony at recording times.

Statistical analysis

We first counted the number of vocalisations per 12-min recording (hereafter reported as vocalisation rate, i.e. number of vocalisations per minute). Since vocalisation rates were not different between the two breeding seasons (Mann–Whitney U-test: $U = 8705.0$, $P = 0.616$), we combined the data for all further statistical analyses.

Since vocalisation rates differed from the normal distribution (Kolmogorov–Smirnov test: $z = 3.431$, $P < 0.001$), we used Spearman Rank correlations to test for the effect of air temperature and Mann–Whitney U-tests to test for the effects of egg category and sibling presence. We examined the change in vocalisation rates between the penultimate day and the last day before hatching with a Wilcoxon signed rank test. Statistical tests were performed in SPSS 15.0.

Results

We made a total of 269 recordings on 196 different eggs (Table 1). Some of the eggs initially included in the study could not be recorded due to egg loss or predation, because they never met the eggshell hole size criterion for recording, or because the two field-workers were unable to record all the hatching eggs simultaneously during the short hatching period (11 days in 2006 and 12 days in 2007). 133 eggs were recorded once (5 only on the penultimate day and 128 only on the last day before hatching) while 68 eggs (38 A-eggs and 30 B-eggs) were recorded on both days. All the recorded chicks subsequently hatched successfully.

Table 1. Number of recordings made by year (2006 or 2007) and egg category (A- or B-egg) on the penultimate day or on the last day before hatching.

		2006	2007	Total
Last day	A-egg	42	50	92
	B-egg	54	50	104
	Total	96	100	196
Penultimate day	A-egg	17	24	41
	B-egg	15	17	32
	Total	32	41	73
Grand total		128	141	269

A-chicks increased the number of vocalisations at lower temperatures on the penultimate day before hatching ($r_s = -0.378$, $P = 0.015$, $n = 41$, Figure 1) but not on the last day before hatching ($r_s = -0.072$, $P = 0.497$, $n = 92$). B-chicks did not significantly change their hatching vocalisation behaviour according to temperature ($r_s = 0.189$, $P = 0.299$, $n = 32$ on the penultimate day before hatching, and $r_s = 0.005$, $P = 0.957$, $n = 104$ on the last day before hatching).

Median vocalisation rates (with interquartile ranges) in A-eggs were 0.33 (0–1.17) and 0.67 (0.08–1.33) vocalisations per minute on the penultimate and the last days before hatching, respectively. The equivalent figures in B-eggs were 0.33 (0.08–0.94) and 0.58 (0.25–1.42) vocalisations per minute, respectively. Hatching vocalisation rates did not differ significantly between A- and B-chicks, either on the penultimate day before hatching ($U = 639.0$, $P = 0.849$, $n = 73$) or on the last day before hatching ($U = 4394.5$, $P = 0.325$, $n = 196$).
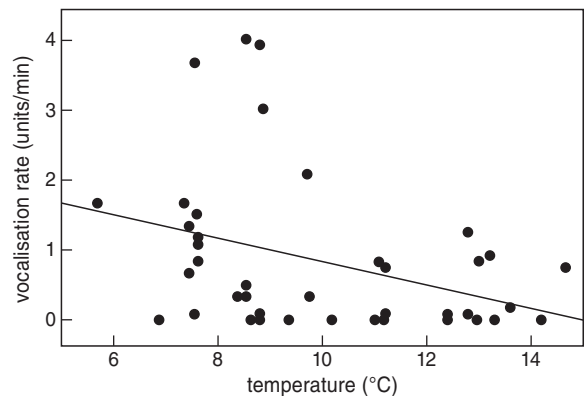

Figure 1. Hatching vocalisation rates (in number of vocalisations per minute) in relation to the ambient temperature for A-chicks on the penultimate day before hatching ($n = 41$).

Hatchlings from A-eggs did not have significantly different vocalisation rates in the presence of a sibling during hatching ($U = 143.0$, $P = 0.200$, $n = 41$ on the penultimate day before hatching and $U = 909.0$, $P = 0.675$, $n = 92$ on the last day before hatching). The same result was observed for hatchlings from B-eggs ($U = 88.5$, $P = 0.134$, $n = 32$ on the penultimate day before hatching and $U = 1200.5$, $P = 0.595$, $n = 104$ on the last day before hatching).

Chicks did not vocalise significantly more or less often during the penultimate day before hatching than during the last day before hatching (medians (inter-quartile ranges) of 0.33(0–1) and 0.58(0.17–1.38) respectively; $z = 1.481$, $P = 0.139$). Air temperatures were also not significantly different between the penultimate day and the last day before hatching (respectively 10.14 ± 2.52°C and 9.77 ± 2.54°C; $z = 1.714$, $P = 0.087$).

Discussion

Both A- and B-chicks of Rockhopper Penguins produced vocalisations during hatching. This observation was expected for this semi-altricial bird since vocalisations usually begin several days before hatching in precocial birds (Gottlieb & Vandenbergh 1968, Oppenheim 1972) and altricial birds also produce vocalisations during incubation and hatching (Evans 1988).

Despite a relatively large range in ambient air temperature (5.6°C to 14.7°C), hatching vocalisation rates were not consistently influenced by temperature. Some chicks can show only a weak incipient vocal response to temperature that begins to increase late in incubation and becomes fully functional only after hatching (Bugden & Evans 1999). As our observations were performed during the last stage of incubation, i.e. hatching, we expected an increase in hatching vocalisation rates at lower temperatures. This was the case neither during the penultimate day nor during the last day before hatching for B-chicks but A-chicks tended to vocalise significantly more often in cold temperatures than in warm temperatures during the penultimate day before hatching. Figure 1 suggests that this statistically significant result was due to a relatively small number of chicks with very high vocalisation rates at low temperatures. We propose that A-eggs, because of their smaller size, should cool more easily than B-eggs and therefore some A-chicks should increase vocalisation rates at low temperatures. However, this result has to be taken with caution since it is not significant when correcting for multiple comparisons. Alternatively, vocalisations may be more vigorous below a temperature threshold, and the range of ambient temperatures experienced in our experiment might have fallen below this threshold. It is therefore not yet totally clear whether hatching vocalisations are a means to solicit parental care in penguins.

We may also hypothesise that during the penultimate day before hatching, chicks were vocalising for heat when necessary, whereas during the last day before hatching, chicks were vocalising to signal their arrival whatever the temperature. Indeed, as A-chicks hatch around 24h after their siblings, they might increase their chance of survival by signalling to their parents that they are viable and will soon hatch. However, this hypothesis would require a general increase in vocalisation behaviour between the penultimate and the last day before hatching. We observed only a modest increase in vocalisation behaviour over this period, which did not reach statistical significance. This result does not support the hypothesis that hatchlings signal their imminent hatching by an increase in vocalisation rates.

A-eggs are significantly more likely to be dislodged or to disappear during incubation than B-eggs, but most loss of A-eggs occurs at hatching (Lamey 1993). However, contrary to our expectations, B-chicks did not vocalise more often than A-chicks during this crucial period. This result suggests that the difference in survival between chick categories is not related to a difference in hatching vocalisation behaviour. It is consistent with the observation that the A-egg produced by Rockhopper Penguins has, when alone, the same theoretical intrinsic potential to lead to a fledged chick as the B-egg (Poisbleau *et al.* 2008).

Although we expected that hatchlings with a sibling would perform more vocalisations than hatchlings without sibling, this was not the case. Two reasons could explain this result. For methodological reasons, hatchlings were recorded alone in the box even if they had a sibling. We hypothesised that the recording time was short enough to maintain the imprinting of their nest environment but this might not have been the case. Hatchlings might adapt their behaviour to the presence of a sibling only when they hear or feel it. On the other hand, hatchlings might not be able to perceive the presence of a sibling and would therefore not be able to adapt their behaviour. Alternatively, we may hypothesise that hatchlings with a sibling did not have more needs than hatchlings without a sibling, despite an apparently lower incubation temperature for small eggs (A-eggs) than for large eggs (B-eggs) in clutches of two eggs in crested penguins (Burger & Williams 1979, Massaro & Davis 2004).

In conclusion, vocalisation rates by hatching

Rockhopper Penguin chicks do not seem to be involved in the higher survival of B-chicks over A-chicks. Hatching vocalisation rates did not increase appreciably between the penultimate and the last day before hatching. They increased only slightly with lower temperatures and only among A-chicks on the penultimate day before hatching. These results suggest that chicks of this species might gain little benefit from increased vocal behaviour at the late incubation stage. However, further behavioural validation of the (potential) effects of hatching vocalisations on parental behaviour would be necessary to fully explore the functional role of hatching vocalisations in penguins; do higher rates of vocalisation lead to increased parental care during or after hatching (e.g. protection against predators, incubation temperature or food provisioning) in this species? It also remains possible that other characteristics of vocalisation behaviour (such as call duration, frequency values or loudness) provide signals for incubating parents , or that these calls mainly serve a function during the mutual learning of individual voices needed for parent–offspring recognition, but are not involved in soliciting parental care.

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Samenvatting

Dit onderzoek beschrijft het stemgedrag (de frequentie van piepen) van jonge Rotspinguïns *Eudyptes chrysocome* (Falkland-eilanden) op de dagen voor het uitkomen. Eieren die het eerst zijn gelegd komen pas na het tweede ei uit, en bovendien produceren ze kleinere jongen die vaker dood gaan dan jongen uit de laatst gelegde eieren. Als het piepen een eerlijk signaal om hulp is aan de ouders, dan is te verwachten dat jongen in het eerste ei vaker piepen dan die in het tweede, en dat ze vaker piepen als het koud is. Het aantal malen dat de jongen geluid maakten was onafhankelijk van de omgevingstemperatuur. Er bestond geen verschil in stemgedrag tussen jongen in de eerste of latere eieren. Er bestond ook geen verschil in geluid tussen de dag van uitkomen en de dag daarvoor. Evenmin had de aanwezigheid van een eerder uitgekomen broer of zus invloed op het stemgedrag. Op grond van de resultaten vermoeden de auteurs dat het piepen door jonge Rotspinguïns rond het tijdstip van uitkomen geen extra ouderzorg opwekt.   (JP)