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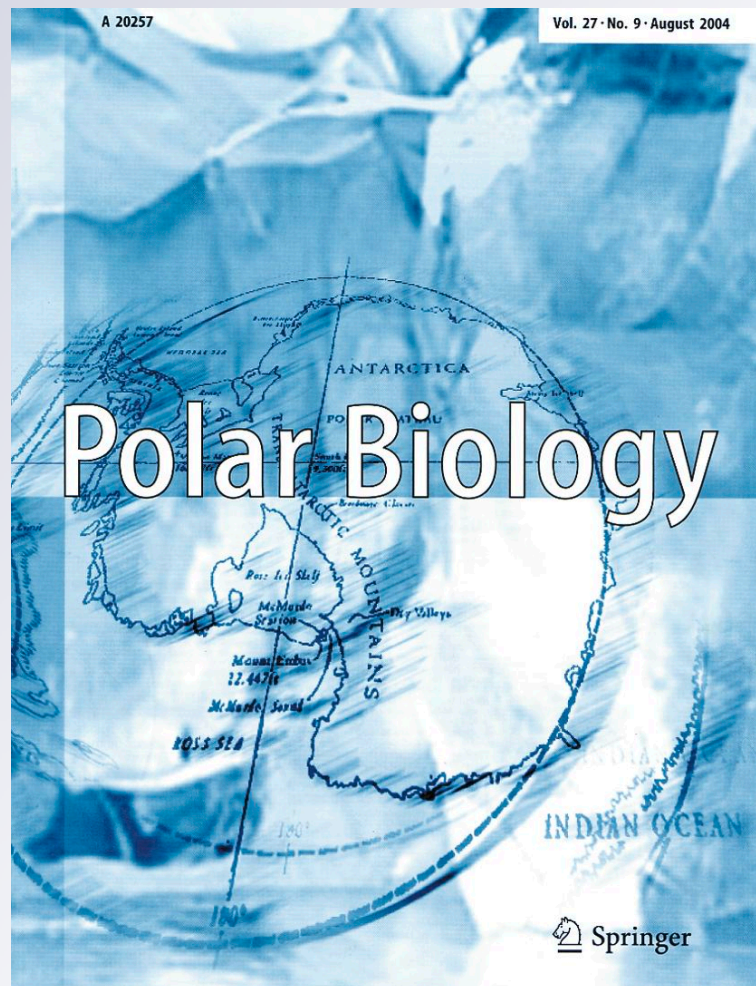
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## Potential mode of clutch size determination and follicle development in *Eudyptes* penguins

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**Abstract** It has long been held that *Eudyptes* penguins will only ever develop a maximum of two mature yolky follicles to match their invariant two-egg clutch, an idea inferred largely from egg removal studies. Combining our own data with those from a previous but rarely cited study and by applying these to a simple developmental model, we show that macaroni penguins (*Eudyptes chrysolophus*) develop up to four large, yolky vitellogenic follicles, and they do so despite the fact that they will never lay more than two eggs or rear more than one chick, a tactic that seems maladaptive given their realized reproductive success. We discuss these results within the context of the usual pattern of reproductive investment in *Eudyptes* penguins and suggest a broader significance to modes of clutch size determination among all penguins (order Sphenisciformes).

**Keywords** Egg follicle · Follicle recruitment · Macaroni penguin · Yolk

### Introduction

Crested penguins (*Eudyptes* spp.) show a unique pattern of reproductive investment among birds (Williams 1995; Davis and Renner 2003): all lay a two-egg clutch but typically only rear a single chick. Additionally, clutches are characterized by extreme intraclutch egg size dimorphism in which the size of the first-laid A-egg is only 56–84% that of the second-laid B-egg. Furthermore, the species with the greatest dimorphism are characterized by early, very high loss of the smaller A-egg, often during laying or by early incubation (Williams 1990; Davis and Renner 2003), which usually involves deliberate egg ejection or “maternal infanticide” by the laying female (St. Clair et al. 1995). In the rare cases where both eggs do hatch, the chick from the second-laid and larger B-egg hatches first, i.e. there is reversed hatching asynchrony to confer a competitive size advantage to the B-chick (St. Clair 1996). In the three most dimorphic *Eudyptes* species, a maximum of 2% of A-eggs survive to hatching, so the single surviving chick is almost always from the B-egg (Williams 1995). Thus, despite two eggs, the *Eudyptes* have a ‘functional’ one-egg clutch. This reproductive pattern is unparalleled in birds (Christians 2002) and has remained unexplained despite over 60 years of interest (Wynne-Edwards 1962; Lack 1968; Williams 1990; St. Clair 1992, 1996, 1998; St. Clair et al. 1995; Davis and Renner 2003). In the context of life history evolution, this reproductive allocation phenotype would appear maladaptive from the perspective of follicle recruitment: the time and energy spent provisioning and producing the A-egg is almost invariably wasted (Williams 1990).

The mechanics of follicle development and clutch size determination in *Eudyptes* penguins is a poorly understood topic (Lack 1947; Paludan 1951; Haywood 1993), and

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most of what we presently know has been inferred from egg removal experiments. When the first and/or second eggs are experimentally removed from their nests when laid, *Eudyptes* penguins will not lay any additional eggs (macaroni penguins, *Eudyptes chrysolophus*, and rockhopper penguins, *E. chrysocome*; Gwynn 1953, and as cited by Haywood 1993). It was therefore concluded that *Eudyptes* would only ever recruit and develop two mature, vitellogenic follicles, i.e. the number of developing follicles equals their two-egg clutch size. Paludan (1951) defined this as a “determinate” mode of clutch size determination. In this determinate 2-egg clutch scenario, the production of the follicle that is destined to become the unhatched egg or unfledged chick seems “wasteful”, as would the production of any *additional* follicles that are neither ovulated, nor oviposited nor destined to become chicks. However, in this study, we present rarely obtained anatomical data from the dissection of gravid female macaroni penguins (*E. chrysolophus*) to show that in fact a hierarchy of up to four large vitellogenic follicles is produced, a result that corroborates data from a previously published but rarely cited study (Gwynn 1993a). Despite the small sample size in this study ( $N = 4$ ), we conclude that macaroni penguins develop more follicles than are needed for their realized clutch size, which suggests an “indeterminate” mode of clutch size determination typical of most other avian species examined for this trait (Haywood 1993). This occurs despite the fact that *Eudyptes* are functionally determinate layers, laying an invariant 2-egg clutch. We discuss the implications of these observations for our general understanding of clutch size determination in penguins at large (order Sphenisciformes).

## Methods

Fieldwork was conducted between October and November 2008 at a large breeding colony of approximately 40,000 pairs of macaroni penguins on Goldcrest Point, Bird Island, South Georgia (54°01'S, 38°02'W), and all research activities conformed to permits issued by the British Antarctic Survey and Simon Fraser University (897B-08). Female macaroni penguins began occupying the breeding colony between 2 and 10 November (median date 6 November), which is consistent with long-term demographic records kept at Bird Island (Williams 1995; British Antarctic Survey unpublished data). Egg laying commenced 7–14 days after arrival, with the mean date of A-egg laying occurring between 15 and 18 November and B-egg laying between 18 and 21 November.

Dead female penguins ( $N = 2$ ) were collected opportunistically during the week prior to peak A-egg laying in an attempt to collect fully developed follicles. Although we

do not know the cause of death of these penguins, they were without any visible internal and external trauma, and patterns of follicle development were nearly identical to those in the healthy laying females ( $N = 2$ ) killed and dissected by Gwynn (1993a) (see Table 1). This suggests that whatever the actual cause of death, it had no apparent bearing on follicle development. Furthermore, the body mass of these penguins (4.88 and 4.90 kg) did not differ from newly arrived, laying females ( $4.97 \pm 0.3$  kg; Crossin et al. 2010). Judging from the warmth and flaccidity of the carcasses and the fact that they had not yet been scavenged, they likely died only minutes to tens of minutes before collection (certainly less than 1 h as carcasses are always quickly scavenged). Necropsies were thus performed, and for each penguin, the total mass of the reproductive tract (ovaries, follicles, and oviduct inclusive) was recorded (nearest 0.1 g), and the mass of all developing follicles was measured individually (nearest 0.1 g and nearest mm).

Using these data on follicle sizes and published data on the composition of macaroni penguin eggs (Gwynn 1993b), we apply these to a previously published model of yolk formation in macaroni penguins (Crossin et al. 2010). In this model, all follicle data were fitted to known temporal sequences of follicle development in macaroni penguins quantified by Grau (1982): the mean interval from initiation of egg formation to A-egg laying is 23.4 days, which includes 16.4 days for yolk formation plus an additional 7 day lag during which albumin and shell constituents are deposited before laying.

## Results

Data regarding the size and number of developing, vitellogenic follicles (e.g. F1–F4 follicles) from the two penguins that we dissected, as well as the two dissected by Gwynn (Gwynn 1993a, b), are presented in Table 1. These data show that female macaroni penguins develop at least four vitellogenic follicles. We should note however that each female also had conspicuous clusters that contained many ( $N > 20$ ) smaller pre-hierarchical follicles in line for potential recruitment. Using these follicle mass data, modelled curves of yolk deposition are presented in Fig. 1. Quadratic curves were fitted to estimates of F1 and F2 follicle masses back-calculated from estimates of final follicle masses (as per Gwynn 1993b, Astheimer and Grau 1990, and Crossin et al. 2010). Because average final masses of F3 and F4 masses are not known as they are never ovulated (i.e. they never complete development and are resorbed shortly after the B-egg is laid), we made the assumption that F3 and F4 follicles would follow the same developmental trajectory as the F2 follicle preceding them.

**Table 1** Attributes of reproductive follicles measured in gravid female macaroni penguins (*Eudyptes chrysolophus*) at Bird Island, South Georgia, and from Heard Island

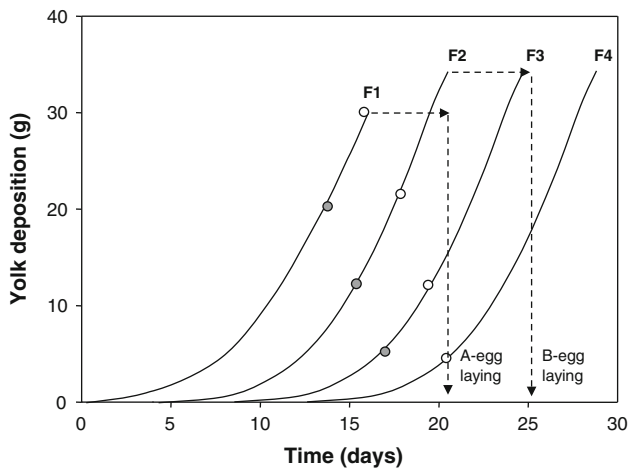
Locale	Penguin number	Follicle	Mass (g)	Diameter (mm)
Bird Island	1	F1	21.5	32.5
		F2	14.2	29.0
		F3	6.9	23.0
		F4	<1.0	8.4
	2	F1	30.5	35.4
		F2	22.6	32.9
		F3	12.3	27.8
		F4	4.0	18.4
Heard Island <sup>†</sup>	H53/B5	F1 <sup>+</sup>	25.6	–
		F2	30.0	39.0
		F3	23.2	36.0
		F4	12.1	29.0
	H53/B6	F1	Already laid	–
		F2 <sup>+</sup>	32.3*	–
		F3	26.3	37.0
		F4	n/a	n/a

n/a not available

\* Follicle development complete, albumin being deposited but shell not yet present

<sup>†</sup> Data from Gwynn 1993a

<sup>+</sup> Follicle in oviduct



**Fig. 1** Modelled rates of yolk deposition in macaroni penguin egg follicles. Final F1 and F2 follicle masses were derived from egg constituent data presented by Gwynn (1993b). To the F1–F4 follicle masses measured from the penguins in Table 1, quadratic curves were fitted from back-calculation of the final follicle mass estimates (see Astheimer and Grau 1990). The theoretical, mature final masses of F3 and F4 follicles were assumed to be the same as that for the F2 follicle preceding them (see “Methods”). Grey circles correspond to the follicle masses of “Penguin 1” in Table 1, which were measured on 9 November 2008, approximately 2 days before the peak of A-egg laying. White circles correspond to follicle masses for “Penguin 2”, which were measured on 12 November 2008, just 5 days before peak A-egg laying

(The F1 follicle follows a different trajectory as it begins developing at sea and is subject to a migratory constraint (see Crossin et al. 2010), unlike the F2 follicle that, like the F3 and F4 follicles, is developed on land.) This assumption appears to be sound as the measured masses of the F3 and F4 follicles were exactly as predicted by the model (Fig. 1) and follow a pattern of follicle recruitment and growth

typical of most other birds, including seabirds (e.g. Astheimer and Grau 1990).

### Discussion

The data that we present in this paper suggest that, at the time of peak egg laying, female macaroni penguins can produce a hierarchy of up to four yolky, vitellogenic follicles (F1–F4 follicles). Applying these data to a simple model of yolk development and growth, we show that these four follicles develop in a predictable sequence in the days leading up to A-egg laying (Fig. 1), an observation that corroborates data from a previously published but rarely cited study (Gwynn 1993a; Table 1). The size of the developing F3 and F4 follicles in this study as well as those presented in Gwynn (1993a) is exactly as predicted assuming a model in which follicles are recruited into vitellogenesis every 3–4 days (as are the F1 and F2 follicles that become the A- and B-eggs) and conforming to the non-linear (i.e. quadratic) growth curve identified by Grau (1982) (see also Astheimer and Grau 1985; Crossin et al. 2010). Collectively, these data dispel the long-standing notion that macaroni penguins produce a maximum of only two well-developed follicles (Williams 1981), which persists despite the publication of Gwynn’s 1993a study. Furthermore, our study highlights the importance of considering the physiology and anatomy of follicle development, because inferences about the mechanisms of clutch size determination in macaroni penguins based simply on the laying response to egg removal experiments can be misleading. Without internal examination, there is no way to determine what is happening at the level of the ovary.

Our results suggest a broader significance to the mechanisms of clutch size determination among all penguins. In his review, Haywood (1993) identified the penguins (Sphenisciformes) as unique among birds as some species possess a “semi-determinate” mode of clutch size determination in which a limited number of follicles are produced beyond those needed for the actual clutch. His conclusion that “at present, the only known cases of semi-determinate laying are found among the penguins” was inferred from egg removal experiments (e.g. Gwynn 1953; Sladen 1958; Taylor 1962; Astheimer and Grau 1985) with no insight on the physiological or anatomical changes underlying the timing and pattern of follicle development. For example, in *Pygoscelis* penguins (Adélie- *Pygoscelis adeliae*, and gentoo- *P. papua*), when eggs are removed from the nest as laid, starting with the first egg, some females will lay a third egg. However, when only the second egg is removed as laid, no extra eggs are laid (Gwynn 1953; Sladen 1958; Taylor 1962; Stonehouse 1963; Astheimer and Grau 1985), and under no circumstance will a fourth egg be laid. This led Astheimer (1985) and Astheimer and Grau (1985) to conclude that only three large yolky follicles ever develop in gravid *Pygoscelis* females, which would fit the semi-determinate mode *sensu* Haywood (1993), though to our knowledge, no systematic examinations of follicle numbers have been made. In contrast, *Eudyptes* penguins will not lay additional eggs in response to egg removal (nor will the single-egg King penguins *Aptenodytes patagonica*; Stonehouse 1960), which led Williams (1981) to conclude that *Eudyptes* would only ever produce two mature follicles to match their invariant two-egg clutch (i.e. a determinate mode *sensu* Haywood [1993]). We have shown that macaroni penguins develop a hierarchy with at least four large vitellogenic follicles, as well as many smaller pre-hierarchical follicles in line for recruitment; they are not determinate layers but must be either semi-determinate or indeterminate layers based on Haywood's (1993) definitions. The correct classification depends on whether all macaroni penguins develop a fixed number of four follicles (semi-determinate) or whether, physiologically, they can produce additional F5 or F6 follicles under some circumstances (indeterminate). We cannot fully resolve this issue since, given the rapid yolk development (RYD) curves and the 4-day interval between recruitment of follicles (Fig. 1; see also Crossin et al. 2010), an F5 follicle would either not yet be recruited or still be very small in penguins bearing a fully developed F1 follicle (see Fig. 1). In our view, the presence of many pre-hierarchical vitellogenic follicles in the ovary suggests that an indeterminate mode of follicle development is more probable. We therefore suggest that, at this stage, it is most parsimonious to conclude that *Eudyptes* are an exception to the

semi-determinate rule governing follicle development in most other penguins. However, more systematic examinations are required before a definitive classification can be made.

If we assume that current data and interpretations of follicle development in *Pygoscelis* penguins are correct (as per Astheimer and Grau 1985, though as noted earlier, no systematic assessments of follicle hierarchies have been made), then from a physiological perspective, our data suggest that the Sphenisciformes have evolved several different mechanisms of clutch size determination: physiologically, the *Eudyptes* may be indeterminate, producing many follicles, while *Pygoscelis* and other two-egg clutch penguins (*Spheniscus*, *Megadyptes*, *Eudyptula*) may be semi-determinate, producing a limited number of additional follicles beyond those needed for a normal clutch size, and finally *Aptenodytes*, which are likely determinate layers with an invariable one-egg clutch.

The fact that macaroni penguins show a pattern of follicle recruitment and growth that is typical of most other avian species, developing more follicles than the number of eggs laid, is especially intriguing in the context of the *Eudyptes*' unique pattern of reproductive investment. The three species with the greatest egg size dimorphism (macaroni *E. chrysolophus*, royal *E. schlegeli*, and erect-crested *E. sclateri*) are “obligate brood reducers” (Lamey 1990) and will eject the smaller A-egg from the nest despite its viability (Williams 1990). Ultimately, nearly all A-eggs are lost prior to or just as the larger B-eggs are laid, and all pairs fledge only a single chick (the B-egg chick) (Warham 1972; Miskelly and Carey 1990; Williams and Croxall 1991; Williams 1995; Davis and Renner 2003). Although some A-egg chicks can hatch and survive to fledging in Snares (*Eudyptes robustus*) and rockhopper penguins (*E. chrysocome*), successful fledging of twins is very rare (up to 2.6% in rockhoppers; Poisbleau et al. 2008). Field observations of these two species show that nearly all surviving chicks are B-egg chicks (Marchant and Higgins 1990; Poisbleau et al. 2008). It is only in the least egg-size-dimorphic Fiordland penguins (*E. pachyrhynchus*) that two chicks are best able to survive to fledging, although the percentage that do so is still comparatively small. There is just one report of 12% of Fiordland pairs rearing two chicks “in one good year” (McLean 2000). Therefore, with the possible exception of *E. pachyrhynchus*, all *Eudyptes* penguins have a functional 1-egg clutch and successfully rear only a single chick despite laying a 2-egg clutch. Our data add yet another counterintuitive element to an enigmatic problem: not only do macaroni penguins lay two eggs while only ever rearing a single chick, but they initiate the development of up to four yolky follicles, which seems even more “wasteful” given their realized reproductive success.

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