

# Sex ratio and fledging success of supplementary-fed Tengmalm's owl broods

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## Abstract

A nest box population of Tengmalm's owls (*Aegolius funereus*) in northern Sweden was studied to investigate the effects of extra food on the sex ratio between hatching and fledging in this sexually size-dimorphic species. The brood size and brood sex ratio of supplementary-fed and control broods were compared. Newly hatched nestlings were blood sampled and sexed by polymerase chain reaction (PCR) amplification of the sex-linked *CHD1Z* and *CHD1W* genes. The brood sex ratio at hatching was strongly male biased (65%); this was also the case in broods where all eggs hatched (72%). There was no relationship between hatch order and sex ratio, and hatching sex ratio did not vary significantly with laying date. Brood size decreased between hatching and fledging, but did not differ between fed and control broods at either stage. Brood sex ratio did not differ between hatching and fledging, and fledging sex ratio did not differ between fed and control broods. It was concluded that, at least during the year in which the study was carried out, feeding had no effect on brood reduction, and that male and female nestlings did not show any differential mortality. The mechanisms behind the male-biased sex ratio at hatching, and any possible adaptive reasons for it, are not known.

**Keywords:** *Aegolius funereus*, avian *CHD1* genes, male-biased hatching sex ratio, mortality, PCR amplification, supplementary feeding

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## Introduction

Fisher (1930) explained theoretically why equal investment in sons and daughters should be an evolutionarily stable strategy. However, if the relative fitness of sons and daughters varies under different circumstances, it may be adaptive for parents to bias investment in favour of one sex (Trivers & Willard 1973; Charnov 1982). There is increasing evidence that avian brood sex ratios are variable and sometimes deviate adaptively from parity with, for example, food supply (e.g. Appleby *et al.* 1997; Kilner 1998), season (e.g. Dijkstra *et al.* 1990; Zijlstra *et al.* 1992) and parental quality (e.g. Ellegren *et al.* 1996; Svensson & Nilsson 1996). Recent developments in molecular sexing of birds have enabled brood sex ratios to be accurately determined from hatching, and allow for detailed sex allocation studies (reviewed by Ellegren & Sheldon 1997).

Sexually size-dimorphic birds are interesting subjects for the study of brood sex ratio variation because, in some

such species, the two sexes have been found to have different energy requirements during growth (e.g. Lindén 1981; Fiala & Congdon 1983; Teather & Weatherhead 1988). The larger sex grows at a faster rate and requires more food than the smaller sex. Fisher's theory of equal investment (Fisher 1930) predicts that parents should bias the offspring sex ratio in favour of the sex that is cheaper to rear (Charnov 1982; Frank 1990). While some studies present biased brood sex ratio data that are consistent with Fisher's theory (e.g. Bednarz & Hayden 1991; Torres & Drummond 1999), in most cases it is more probable that such brood sex ratio bias is a result of non-adaptive mortality differences between the sexes, which have different energy requirements (Clutton-Brock *et al.* 1985; Teather & Weatherhead 1988; Weatherhead & Teather 1991). Many studies of sexually size-dimorphic birds provide evidence for this (e.g. Røskaft & Slagsvold 1985; Griffiths 1992; Torres & Drummond 1997).

Tengmalm's owl (*Aegolius funereus*) is a small, nocturnal predator that is common in boreal Scandinavia. It breeds readily in nest boxes and is therefore relatively easy

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to study. Tengmalm's owl feeds mainly on voles (e.g. Korpimäki 1981, 1988; Mikkola 1983; Hörnfeldt *et al.* 1990), which in northern Sweden undergo 3–4-year population cycles of high amplitude (Hörnfeldt 1978, 1994; Hörnfeldt *et al.* 1986), which affects much of the breeding biology of Tengmalm's owl, e.g. laying date, clutch and brood size (Hörnfeldt *et al.* 1990). To our knowledge, brood sex ratio has not previously been studied in this species, and studies of other species with as large an interannual variation in food supply are limited (see Torres & Drummond 1997, 1999 for one such species). In common with most other birds of prey, Tengmalm's owl exhibits 'reversed' sexual size dimorphism, the female being  $\approx 50\%$  heavier than the male during the breeding season (Mikkola 1983; Hakkarainen & Korpimäki 1991) and, hypothetically, if there is a similar relationship among nestlings, differential mortality of male and female nestlings could be expected.

In this study, we present hatching sex ratio data from Tengmalm's owl broods in which newly hatched nestlings were sexed by polymerase chain reaction (PCR) amplification of the sex-linked chromo-helicase-DNA binding (*CHD1*) *CHD1Z* and *CHD1W* genes. We also present the results of a feeding experiment designed to test for any change in brood sex ratio if there was a sex-biased nestling mortality, by comparing supplementary-fed broods with controls.

## Materials and methods

### General fieldwork

The study area was situated in Västerbotten, northern Sweden ( $\approx 64^\circ\text{N}$ ,  $20^\circ\text{E}$ ). This area, dominated by managed coniferous forest with small areas of low-intensity agriculture, is located within the middle boreal vegetation zone (Ahti *et al.* 1968). During 1998, 272 nest boxes were inspected every 3–4 weeks, from March to June, to detect breeding Tengmalm's owls. Nest boxes were of a standard size ( $20 \times 20$  cm base, 8.5 cm entrance hole diameter), and placed along roads at intervals averaging 1 km. The laying date of each detected clutch was estimated by backdating. If the nest was discovered during laying, a laying interval of 2 days per egg was used (Korpimäki 1981). If the nest was discovered later, the wing-length of the oldest nestling was measured to determine its age, and an incubation period of 29 days was assumed (Carlsson & Hörnfeldt 1994). Nests were revisited when hatching was expected (28–30 days after laying; Korpimäki 1981). Data were collected on clutch size, brood size and hatching order, and nestlings were ringed, initially with coloured plastic rings and subsequently with standard metal rings. Nestling mortality was recorded by frequent monitoring and after fledging by X-raying nest box remains to detect rings. A 50- $\mu\text{L}$  blood sample was obtained from all nestlings,

usually 1–3 days after hatching (in a few cases up to 1 week later) by brachial vein puncture, to determine their sex (see below). Blood samples were taken, under licence, from 196 nestlings (in 36 broods), and no ill effects were recorded. Sampling was avoided during bad weather. Blood samples were stored in SET buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, pH 8.0) which was kept cool (below  $10^\circ\text{C}$ ) during the rest of the day in the field and then frozen at  $-70^\circ\text{C}$ .

### Feeding experiment

Sixteen randomly assigned broods (on average every second brood discovered) were provided with additional food during the entire nestling period (from when the first nestling hatched until the last nestling fledged). Broods were fed by placing dead, dark-coloured laboratory mice in the nest box every other day, averaging 100 g per day. This is a reasonable simulation of the natural situation, in which the male deposits dead prey in the nest hole for the female to distribute amongst the nestlings (Korpimäki 1981, 1987). Similar food provision experiments have been performed previously on Tengmalm's owl, before and during laying, to manipulate laying date and clutch size (Korpimäki 1989a; Hörnfeldt & Eklund 1990). The few mice that were not eaten were removed from the nest box each time new ones were provided. The remaining 20 broods acted as controls and were monitored in an identical manner to the fed broods. Fed and control broods were evenly distributed both spatially and temporally. Laying date, clutch size, brood size and brood sex ratio at hatching did not differ between fed and control groups (verified afterwards by *t*-tests). As we focused on comparing the brood sex ratio at hatching with that at fledging, broods that were abandoned by the parents before hatching was complete (three fed and three control broods), and broods in which more than one egg failed to hatch (two fed and four control broods), were not included in any analyses.

### Molecular sexing

Molecular sexing relied on PCR amplification of the sex-linked *CHD1* genes, i.e. *CHD1W* and *CHD1Z*, which map to the avian W and Z chromosomes, respectively (Ellegren 1996; Griffiths *et al.* 1996; Griffiths & Korn 1997; Fridolfsson *et al.* 1998). DNA was prepared by Chelex extraction (Walsh *et al.* 1991) from 3  $\mu\text{L}$  of blood. One per cent of the extraction was used for PCR, essentially carried out as described in Fridolfsson & Ellegren (1999). All reactions were performed in 10- $\mu\text{L}$  volumes containing 0.05 U of *AmpliTag* (Perkin-Elmer), 200  $\mu\text{M}$  of dNTPs, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.75 mM  $\text{MgCl}_2$  and 2 pmol of primers 2550F (5'-GTTACTGATTTCGTCTACGAGA-3')

and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3'). Cycling conditions (in a Perkin-Elmer 9600 thermal cycler) consisted of an initial denaturing step at 94 °C for 2 min, then a 'touch-down' thermal profile of 10 cycles of 94 °C for 30 s, 60–50 °C (1 °C reduction on each subsequent cycle) for 30 s and 72 °C for 40 s. Finally, an additional 27 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 40 s were run. PCR products were separated in 2% agarose gels and detected by ethidium bromide staining. Females were characterized by displaying a *CHD1W*-specific fragment (1.2 kb in size) plus a shorter *CHD1Z*-specific fragment (0.7 kb), while males showed only the shorter Z-fragment.

### Statistical analysis

Statistical analysis was performed using *SPSS*, version 8.0. Sex ratio data (denoted throughout as percentage of males) were divided by 100 then arcsine-transformed before testing. All tests were two-tailed, and the critical threshold for significance was 0.05.

## Results

### Brood sex ratio at hatching

The mean brood sex ratio at hatching was 65% male ( $n = 24$  broods), which was significantly different from an equal (50%) sex ratio (one-sample  $t$ -test:  $t_{23} = 3.29$ ,  $P = 0.003$ ). The mean sex ratio of broods in which all eggs hatched ( $n = 11$ ) was 72% male, also significantly different from equality ( $t_{10} = 2.94$ ,  $P = 0.01$ ). The mean sex ratio of broods in which one egg did not hatch ( $n = 13$ ) was 60% male, but this did not differ significantly from equality ( $t_{12} = 1.77$ ,  $P = 0.10$ ). Mean sex ratio of the earlier (first to third) hatched nestlings ( $n = 90$ , 69%) did not differ significantly from that of later (fourth to eighth) hatched nestlings ( $n = 77$ , 68%) ( $t$ -test:  $t_6 = -0.28$ ,  $P = 0.79$ ). There was no correlation between hatching sex ratio and laying date (Spearman's rank correlation:  $n = 24$ ,  $r_s = -0.07$ ,  $P = 0.73$ ), and the mean hatching sex ratio of 'early' broods (laying date before and including April 9th;  $n = 12$ , 59%) was not significantly different from that of 'late' broods (laying date after April 9th;  $n = 12$ , 72%) ( $t$ -test:  $t_{22} = -1.23$ ,  $P = 0.23$ ).

### Feeding experiment

The effect of supplemental feeding between hatching and fledging on the brood size and brood sex ratio was analysed using a repeated-measures analysis of variance (ANOVA). Brood size or brood sex ratio was used as the dependent variable. Stage (i.e. hatching or fledging) was used as the within-subject factor, and experimental treatment (fed or control) was used as the between-subject

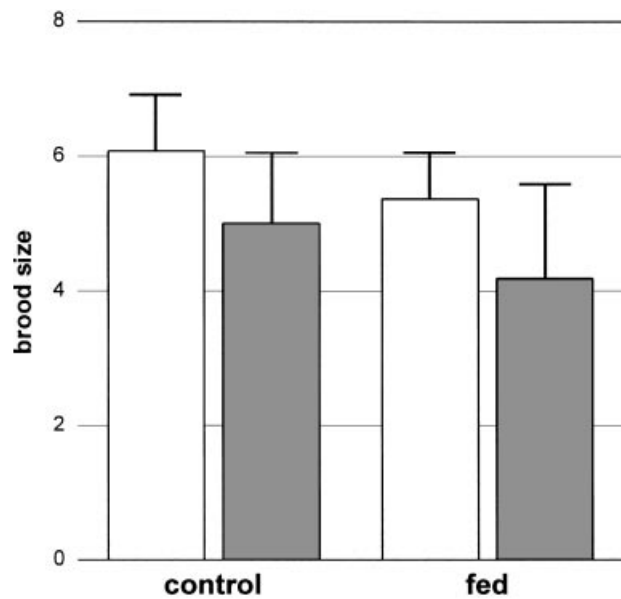


Fig. 1 Mean brood size in control ( $n = 13$ ) and supplementary-fed ( $n = 11$ ) broods at hatching (white bars) and fledging (grey bars). Error bars represent 95% confidence intervals.

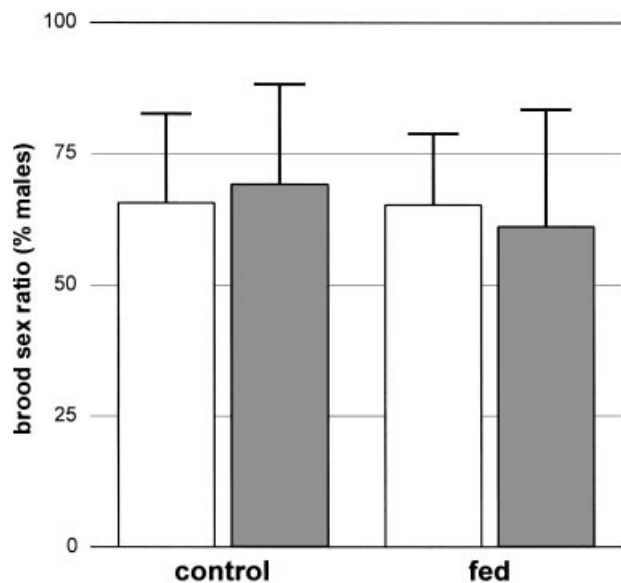


Fig. 2 Mean brood sex ratio (per cent males) in control ( $n = 13$ ) and supplementary-fed ( $n = 11$ ) broods at hatching (white bars) and fledging (grey bars). Error bars represent 95% confidence intervals.

factor. The interaction between stage and treatment was used to detect the effects of supplemental feeding. Brood size decreased between hatching and fledging ( $F_1 = 13.77$ ,  $P = 0.001$ ; Fig. 1), but there was no counteractive effect of supplemental feeding ( $F_1 = 0.03$ ,  $P = 0.86$ ; Fig. 1). Brood sex ratio did not change between hatching and fledging ( $F_1 = 0.53$ ,  $P = 0.47$ ; Fig. 2), and there was no effect of supplemental feeding ( $F_1 = 0.31$ ,  $P = 0.58$ ; Fig. 2).

Overall nestling mortality was 20% for males (18 out of 91 nestlings died) and 19% for females (nine of 47). In fed broods, mortality of male and female nestlings was 24% (nine of 38) and 19% (four of 21), respectively, while in control broods, male and female mortality was 17% (nine of 53) and 19% (five of 26), respectively.

## Discussion

### *Brood sex ratio at hatching*

The sex ratio of Tengmalm's owl broods at hatching was strongly male biased. While this is a notable result, and probably serves some adaptive purpose, we cannot yet explain it. Skewed offspring sex ratios are adaptive if they favour the sex that benefits most from the current environmental situation (Trivers & Willard 1973). A number of recent studies on raptors have reported a seasonal trend in brood sex ratio, with early broods exhibiting sex ratios biased towards the sex most benefiting from this (Daan *et al.* 1996; Smallwood & Smallwood 1998). For example, for European kestrels (*Falco tinnunculus*), the probability of males breeding as yearlings decreased with birth date. The birds appeared to adapt to this as early broods were male biased, whilst later broods were female biased (Dijkstra *et al.* 1990). The same pattern was observed in lesser kestrel (*F. naumanni*) broods (Tella *et al.* 1996). The seasonal trend in brood sex ratio in marsh harriers (*Circus aeruginosus*), was reversed, with female-biased sex ratios early in the season, and male-biased broods later because, in this species, the female's probability of breeding as a yearling decreased with birth date (Zijlstra *et al.* 1992). No significant seasonal trend in brood sex ratio was detected in Tengmalm's owl. However, because of the large interannual variability in cyclic vole supply and breeding success of Tengmalm's owl in northern Sweden (e.g. Hörnfeldt *et al.* 1990; Hörnfeldt 1994), it seems equally possible that any brood sex ratio variation is linked to the phase of the vole population cycle. Tawny owl (*Strix aluco*) broods on territories with more abundant prey (voles) were female biased at hatching. Females born on such territories produced a greater number of nestlings than those on territories with less abundant prey. In contrast, male reproductive success was not affected by prey abundance on their natal territory (Appleby *et al.* 1997). In Montagu's harriers (*C. pygargus*), whose breeding success correlated with vole abundance, no relationship was found between brood sex ratio at fledging and interannual variation in vole abundance (Leroux & Bretagnolle 1996).

### *Feeding experiment*

The eggs of Tengmalm's owl hatch asynchronously, and

the last-hatched nestlings frequently die from a combination of starvation and sibling aggression (Mikkola 1983). Food provision during the nestling stage usually improves survival and production of young (Mock *et al.* 1987; Simons & Martin 1990; Richner 1992; see Newton 1998 for a recent review). In our study, the mean brood size decreased between hatching and fledging, but mortality was not reduced in fed broods. On the other hand, voles were abundant during 1998, which was the first year after the increase year (i.e. the year with the highest population growth rate in summer; *sensu* Hörnfeldt 1994) in the current vole population cycle (data from the Swedish National Environmental Monitoring Programme; B. Hörnfeldt, unpublished). Therefore, the natural food situation could have been too good to reveal any effects of supplemental feeding (cf. Boutin 1990; Newton 1998). Indeed, in many food addition experiments (reviewed by Boutin 1990), subsequent increases in brood size generally do not exceed brood sizes attained under good breeding conditions. Moreover, supplemental feeding does not necessarily reduce sibling aggression (Mock *et al.* 1987), so death of nestlings may have continued despite the plentiful food supply in both fed and control broods.

Brood sex ratio did not change between hatching and fledging, and mortality of male and female nestlings was similar. Supplementary feeding had no effect on brood sex ratio, but this was not surprising in this case because feeding had no effect on mortality in general. Despite sexual size dimorphism at adulthood, the mass of male and female nestlings did not appear to differ to any great extent just before fledging (T. Hipkiss, unpublished). If size dimorphism at the nestling stage is indeed only slight, then any mortality difference would probably be small, although perhaps more pronounced when vole abundance is low or declining. In asynchronously hatching species, mortality differences between male and female nestlings may be of less importance than the mortality difference between older and younger nestlings owing to sibling competition (cf. Bortolotti 1986a,b).

Tengmalm's owl females may continue to grow for a longer period after fledging than males, and increased growth rate and energy requirements might cause them to suffer higher mortality at this later stage. However, we have no information on survival of our broods during the postfledging stage. This period is crucial, as the fledglings must learn to hunt for themselves and accumulate fat reserves for winter (Korpimäki & Lagerström 1988). Males, which have to seek and defend a territory during winter (e.g. Korpimäki 1989b), may themselves suffer higher mortality than females.

Our observation of the male-biased hatching sex ratio is interesting and has not been reported previously in Tengmalm's owl. Little is known about the possible physiological mechanisms whereby female birds (the

heterogametic sex) might adjust the primary sex ratio of their broods (see Krackow 1995 for a review), although an increasing number of studies suggest that this occurs. Tengmalm's owl brood sex ratios should be investigated further, particularly in relation to variation of food supply and prospective breeding success of offspring reared at different stages of the vole population cycle, to establish an adaptive explanation for the male-biased sex ratio presented here.

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Birger Hörnfeldt's research group studies cyclic vole populations and the effects of this variation of food supply on the ecology of Tengmalm's owl. Research in Hans Ellegren's laboratory focuses on general questions concerning evolutionary genetics and molecular ecology. This study forms part of Tim Hipkiss' PhD work.

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