Sex-ratio and body size of sandpiper chicks at Zackenberg, north-east Greenland in 2003

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There is growing evidence that female birds may adaptively bias the sex ratio of their young as a function of environmental conditions. Data on brood sex ratio in shorebirds are scarce, however. In this study we report the brood sex ratios and morphometrics of Arctic sandpipers. Sex was determined in 13–64 chicks of Sanderling *Calidris alba*, Red Knot *Calidris canutus islandica*, Dunlin *Calidris alpina arctica*, and Ruddy Turnstone *Arenaria interpres* in NE Greenland during the 2003 breeding season. Brood sex ratios were biased significantly towards males in Dunlin and Ruddy Turnstone, but in Dunlin this bias disappeared in chicks older than two days. There was a non-significant bias towards females in Sanderling and Red Knot. Only for bill length in Dunlin hatchlings, there were significant differences between males and females. Surprisingly, in contrast to their parents, male chicks had longer bills than female chicks.

INTRODUCTION

It is difficult, if not impossible, to reliably determine the sex of monomorphic shorebird species on the basis of plumage characteristics or size. This certainly is the case for small downy chicks. With the advent of modern molecular techniques, sex can be determined on the basis of DNA retrieved from the red blood cells in small blood samples (e.g. Griffiths *et al.* 1996). This development has resulted in interesting reports on biased sex ratios in adult populations of species with little or no sexual dimorphism. For example, using molecular assays, Nebel *et al.* (2000) detected a near absence of males in Red Knots *Calidris canutus canutus* of the Siberian breeding population during their southward stopover in the Dutch Wadden Sea. In a catch of 90 Red Knots of the American subspecies (*Calidris canutus rufa*) during northward migration in Lagoa do Peixe, Brazil, molecular determination of sex revealed a strong bias towards males. Almost two months later, at the end of the staging period on the next main stop-over in Delaware Bay, USA, the proportion of male Red Knots did not differ from 50%, suggesting that male and female Red Knots synchronise their migration schedules the closer they approach the breeding grounds (Baker *et al*. 1999).

Different from the examples above, in which biases in sex-ratio of a population at stop-over sites are the result of different migration strategies between sexes, wader populations might show sex ratios deviating from 50:50 due to biases in the primary (or brood) sex ratio of birds (Clutton-Brock 1986). To interpret sex ratios of adult shorebirds in different contexts, we require data on the (variation in the) primary sex ratios (i.e. sex ratio of a clutch at egg-laying).

Why would birds skew the sex-ratio of their offspring? When selection pressures act differently on males and females, it might be adaptive for parents to adjust the sex-ratio of their offspring in response to these selection pressures (Trivers & Willard 1973; Sheldon, 1998). In other words, if their sons rear more young during a lifetime or have a higher survival than their daughters under given environmental conditions, females are expected to produce more sons, and *vice versa*. Females might, for example, adjust primary sex-ratio in response to the attractiveness of their partner (Sheldon *et al.* 1999) or depending on systems of parental care (Hasselquist & Kempenaers 2002).

Székely *et al.* (2004) studied sex ratios in a large number of broods of Kentish Plovers *Charadrius alexandrinus*, and found that, although mating opportunities between males and females differed (Székely *et al.* 1999), primary sex ratio was not biased. Published information on brood sex ratios in sandpiper species is rare, however (see Thuman *et al.* 2003 for an exception). This is somewhat surprising in view of the well-studied diversity in life histories, ecology and sexual size dimorphism in waders. Moreover, the invariable clutch size of four eggs and precociousness of the chicks (with little parental care) allows relatively uncomplicated analyses. Here we present data collected during a season of fieldwork

at a High Arctic location in NE Greenland. We obtained a fair series of blood samples of chicks in a number of species (Sanderling *Calidris alba*, Red Knot *Calidris canutus islandica*, Dunlin *Calidris alpina arctica* and Ruddy Turnstone *Arenaria interpres*) to document the sex ratios of chicks of these species for the first time. Having determined sex, we additionally performed an analysis of possible sex differences in hatchling size and mass.

METHODS

Study site and subspecies concerned

The study was carried out at the Zackenberg Research Station on Wollaston Forland (74°28'N, 20°34'W) in NE Greenland. Here, standardised and intensive ecological monitoring started in 1996 (Meltofte 2001, Meltofte & Berg 2003). In addition to the yearly intensive monitoring work, Zackenberg Ecological Research Operations (ZERO) support supplementary studies on particular biological, climatological or geomorphological subjects.

The Dunlins in NE Greenland belong to the subspecies *Calidris alpina arctica*, a relatively uncommon race that purportedly winters in W Africa (Greenwood 1986; Engelmoer & Roselaar 1998). The Red Knots studied at Zackenberg belong to the *islandica* subspecies (Piersma & Davidson 1992, Davidson & Wilson 1992). For Sanderlings and Ruddy Turnstones breeding in NE Greenland no subspecific assignments have been made. However, it is likely that the majority of the Sanderlings winter in W Africa (Gudmundsson & Lindström 1992, Lyngs 2003).

Fieldwork

Daily, from 17 June to 5 August 2003, we searched for shorebird nests and later for parents with chicks in the Zackenberg area, particularly in the 4 km² core study area on the slopes of the Aucellabjerg mountain (see Piersma *et al.* MS). Nests were searched by following alarming adults back to their nests and by dragging ropes of 20–50 m over the tundra surface between pairs of observers to temporally disturb tightly sitting birds from their nests. The latter technique had proven useful in finding nests of Red Knot during earlier expeditions to Siberia (Tulp *et al.* 1998), and helped us to find Sanderling nests as well (Piersma *et al.* MS).

Upon encountering a nest, we marked the nest site with a GPS and placed a white nylon stick 10 m away from the nest in variable direction (but always directly away from the Research Station). This enabled us to relocate the nest on later visits. At first discovery, the eggs were floated in mildly warm water and the angle of the axis of the eggs with either the bottom or the water surface was recorded, as well as the elevation of the eggs above the surface when floating. This technique provides a reliable estimate of hatching date (van Paassen *et al.* 1984, Meltofte & Berg 2003). Eggs were dried off after floating and placed back into the nest cup with a small paper towel to avoid spreading the scent of our fingers onto the eggs and possibly attracting mammalian predators to the nest. We usually tried to catch and ring one or both of the parents. Nests were visited regularly to quickly check for predation and to fine-tune the expected date of hatching by looking for cracks in the egg shell.

We always tried to find the chicks on the day of hatching when they were still immobile and present in the nest or in

Fig. 1. Weighing a three-day-old Red Knot chick on a small electronic scale. (Photo: Jeroen Reneerkens).

the close vicinity. Like most precocial shorebirds, the chicks usually leave the nest several hours after hatching. Sometimes we encountered broods of mobile chicks several days old in our core study area, which were guarded by (unringed) parents which we had not encountered before. The age of these chicks was estimated on the basis of calibrated average growth curves (mass or size; Meltofte & Berg 2003).

The birds were ringed with a steel ring of the Danish ringing centre, weighed to the nearest 0.1 g on an electronic scale (see Fig. 1), and length of tarsus and bill were measured to the nearest 0.1 mm. A tiny droplet of blood was sampled in a capillary by carefully puncturing the leg vein, or the brachial wing vein in older chicks with more developed wings. Blood was immediately stored in 96% ethanol at –20°C in which it was kept until DNA was extracted for analysis in the laboratory.

Molecular sexing

DNA was extracted from the red blood cells with the "GenElute Mammalian Genomic DNA Kit" (Sigma) according to the protocol provided by the manufacturer.

The methods described by Griffiths *et al.* (1996), using P2 and P3 primers provided the basis for the molecular sexing of the sandpiper chicks. This method has been verified for Red Knots (Baker *et al.* 1999). By using primers P2 and P8 (as in Griffiths *et al.* 1998), the method was slightly modified. This is currently the technique we use at the Royal Netherlands Institute of Sea Research to sex sandpipers. PCR products were analysed in 2.5% NuSieve GTG Agarose after electrophoresis for 200 minutes at 60V and staining with ethidium bromide.

Sex ratio analyses

Sex ratio of the different species was analysed (1) for all sampled chicks and juveniles, independently of age or completeness of the brood, i.e. after potential losses by predation or by other unknown causes, (2) with the restricted number

Table 1. Sex ratios of chicks of four shorebird species at Zackenberg, North-east Greenland, in July 2003. Chicks from incomplete broods are included in this table.

Species	Age of pulli	N males	N females	% males
Sanderling *Calidris alba*				
	0–2 days	15	27	38.2
	>2 days	14	8	53.3
Total		29	35	45.3
Red Knot *Calidris canutus islandica*				
	0–2 days	2	6	25
	>2 days	4	1	80
Total		6	7	46.2
Dunlin *Calidris alpina arctica*				
	0–2 days	19	10	65.5
	>2 days	3	4	42.9
Total		22	14	61.1
Ruddy Turnstone *Arenaria interpres*				
	0–2 days	11	4	73.3
	>2 days	6	1	85.7
Total		17	5	77.3
All species		74	61	54.8

of chicks of complete clutches at the day of hatching only. Because none of the encountered broods of two days old or younger was incomplete, these broods had thus not been influenced by possible differences in survival of male or female chicks. We therefore decided to include these broods (one Dunlin, one Ruddy Turnstone, one Sanderling and two Red Knot broods) in the analysis of brood sex ratio. Therefore, in practice there was no difference between what is strictly defined as the primary sex-ratio (i.e. the egg sex ratio) and the secondary sex ratio (sex-ratio of a recently hatched brood).

We sampled blood droplets of 135 chicks and juveniles in total. Sixty of these belonged to complete clutches of four chicks sampled at hatching date (four Dunlin, two Ruddy Turnstone and nine Sanderling broods). However, for completeness, we also present data on the size and mass of all chicks up to an estimated age of two days (n = 91), including those from clutches where not all chicks were sampled.

We used a chi-square goodness of fit test to determine whether the number of males in complete broods deviated significantly from the expected two. A binomial test was used to determine whether the ratio males:females amongst all sampled chicks within a species deviated from the expected 1:1 ratio. The statistical in the chi-square test is a complete brood, in the binomial test it is an individual chick or juvenile.

RESULTS

Sex ratios

The sex ratios of the hatchlings differed between species and are shown in Table 1. The sex ratio in 10 complete Sanderling broods (40 chicks) was on average 0.35 ± 0.13 males, and did not differ significantly from parity (chi-square test, $\chi^2_4 = 8.67$, p < 0.10). The sex ratio (0.44 male) of all 63 Sanderling chicks and juveniles, including those from incomplete clutches, was also not biased (binomial test, p = 0.45). We encountered two Red Knot broods that both contained only one male. Brood sex ratio did not differ from parity (chi-square test, $\chi^2_4 = 4.5$, p < 0.5), but with only two broods the power of this test is very low.

The proportion of males in complete broods of freshly hatched Dunlins was 0.75 ± 0.25. The distribution of males in these broods differed from a binomial distribution (chi-square test, $\chi^2_4 = 10.73$, p < 0.05). If we include all Dunlin chicks and juveniles from which we obtained blood samples during the breeding season of 2003 (n = 33), the sex ratio (0.61 males) was not significantly different from parity (binomial test, p = 0.163).

Sex ratio of the three complete Ruddy Turnstone broods was significantly male-biased (0.66 males, chi-square test, $\chi^2_4 = 18.56$, p < 0.001), although, as with the two Red Knot broods, the statistical power of these tests is low in view of the small sample size. Of all 22 new-born turnstones from which we obtained blood samples, 17 were male (ratio 0.77), which deviates from the expected 0.50 (binomial test, p = 0.017).

Hatchling body sizes

The two Red Knot nests found were depredated before hatching by, most likely, an Arctic Fox *Alopex lagopus* and a Wolf *Canus lupus*, respectively. Thus, we cannot show data of hatchling mass or biometrics of this species. Body mass or tarsus length did not differ between the sexes of Dunlin, Sanderling and Ruddy Turnstone (Table 2). Interestingly, bill length of male Dunlin hatchlings (all measured on the day of hatching) was significantly larger than that of females. In

Table 2. Mass and size (± standard deviation) at hatch of chicks of four shorebird species at Zackenberg, NE Greenland, in July 2003. We include measurements of chicks up to an estimated age of two days.

Species	Sex (n)	Body mass (g)	Bill length (mm)	Tarsus length (mm)
Sanderling *Calidris alba*	Males (15)	7.23 ± 0.43	9.19 ± 0.57	20.5 ± 0.86
	Females (27)	7.31 ± 0.44	9.35 ± 0.69	20.9 ± 0.74
	t-test	t = 0.534, p = 0.58	t = 0.823, p = 0.42	t = 1.559, p = 0.13
Dunlin *Calidris alpina*	Males (17)	6.65 ± 0.48	8.55 ± 1.64	20.6 ± 0.78
	Females (8)	6.65 ± 0.67	6.79 ± 1.66	21.1 ± 0.61
	t-test	t = –0.012, p = 0.99	t = –2.497, p = 0.002	t = 1.766, p = 0.09
Ruddy Turnstone *Arenaria interpres*	Males (11)	11.0 ± 1.20	11.0 ± 0.77	21.9 ± 0.68
	Females (4)	11.5 ± 0.38	10.7 ± 0.56	21.5 ± 1.14
	t-test	t = 1.414, p = 0.27	t = –0.639, p = 0.53	t = –0.631, p = 0.56

fully-grown Dunlins, it is the females that have larger bills than the males (Prater *et al.* 1977, Engelmoer & Roselaar 1998). Bill length did not differ between the sexes in Sanderling and Ruddy Turnstone (Table 2).

DISCUSSION

To the best of our knowledge a study by Thuman *et al.* (2003) on sex allocation in Ruffs *Philomachus pugnax* is the only published report on brood sex-ratio and sex-specific hatchling body size in a sandpiper species. We hope that the present study provides further encouragement for others to add to the small, but hard-won, datasets, so that robust patterns may be detected at some future date. Although our dataset is too limited for definitive statements on primary sex ratio bias in the four sandpiper species investigated, it is interesting that we found a male-biased sex ratio in Dunlin broods and also that bill length of male Dunlin hatchlings was larger than those of the females. More extensive studies will be needed to determine whether these patterns are real and biologically meaningful, and to what geographical and temporal extent the strong male bias in the sex ratio of Ruddy Turnstones is upheld. As the amount of bias in the sex-ratio of birds is expected to be small, large datasets will be required (e.g. Sheldon 1998). By expanding the dataset in the future, we might also gain statistical power to study the effects of environmental factors, such as laying date, condition of the parents and insect abundance, on brood sex ratios.

In polygynous species with large variation in reproductive success between males, primary sex ratio adjustments related to the quality of the father and/or mother are especially to be expected (Komdeur & Pen 2002; Hasselquist & Kempenaers, 2002). The lek-breeding Ruff is a shorebird that shows large inter-individual variation in the mating opportunities of males (Van Rhijn 1991). Because female Ruffs are more or less unrestricted in their mate choice, and because there is no paternal care in Ruffs, adjustments in primary sex ratio would be a way for females to influence the fitness returns of their reproductive effort. In two of three study years, primary sex ratio in the population of juveniles was even when the body condition of the females was relatively good (n = 64 and 102 chicks; Thuman *et al.* 2003). However, it was biased towards daughters in the one year in which adult females were in overall poor body condition (n = 120 chicks; Thuman *et al.* 2003). Surprisingly, however, and contradicting the between-year trend, females in good body condition produced more daughters in the 'poor' year than females in poor condition.

Unlike Ruffs, the four sandpiper species investigated by us are thought to be monogamous and extra-pair copulations have, as far as we know, not been shown to occur. Nevertheless, there is evidence that Sanderling females might produce two broods in a single breeding season (Parmelee & Payne 1971). This so-called "double-clutching" offers opportunities for extra-pair copulations and thus for inter-individual variation in mating opportunities such as in Little Stints *Calidris minuta* and Temminck's Stints *Calidris temmenckii* in which females also produce double clutches (Hildén 1983; Breiehagen 1989).

Shorebirds are also interesting study subjects for sex allocation studies because survival chances could differ between sexes e.g. due to different migration strategies (as in Red Knots from the Siberian breeding population (Nebel

et al. 2000); although such survival differences were not apparent in the preliminary analysis by Brochard *et al.* (2002)) and/or latitudinal segregation of sexes during winter (as in first-winter Sanderlings (Meyer 1981), or Western Sandpipers *Calidris mauri* (Nebel *et al.* 2002)). A reproductive bias towards one sex could enhance individual fitness in such systems. Some shorebird species show a large sexual size dimorphism (e.g. Bar-tailed Godwit *Limosa lapponica*) that might involve differences in costs of producing sons and daughters that could also favour primary sex ratio biases (Hasselquist & Kempenaers 2002).

We believe that future research efforts on migration and reproductive strategies of shorebirds that include an examination of sex bias would greatly help to understand the biology of these species.

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Rope-dragging across the tundra helped us to find the nests of Sanderlings and Red Knots.

The tundra near Zackenburg.

Theunis Piersma taking a droplet of blood from a Red Knot chick.