

# Sex ratio varies with egg investment in the red-necked phalarope (*Phalaropus lobatus*)

Willow B. English · Douglas Schamel · Diane M. Tracy · David F. Westneat · David B. Lank

Received: 31 December 2013 / Revised: 21 August 2014 / Accepted: 25 August 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** Fisher's sex ratio theory predicts that on average parents should allocate resources equally to the production of males and females. However, when the cost/benefit ratio for producing males versus females differs, the theory predicts that parents may bias production, typically through underproduction of the sex with greater variation in fitness. We tested theoretical predictions in the red-necked phalarope, a polyandrous shorebird with sex-role reversal. Since females are larger and therefore potentially more expensive to produce and may have greater variation in reproductive success, we predicted from Fisher's hypothesis a male bias in population embryonic sex ratio, and from sex allocation theory, female biases in the clutches of females allocating more resources to reproduction. We measured eggs and chicks and sexed 535 offspring from 163 clutches laid over 6 years at two sites in

Alaska. The embryonic sex ratio of 51.1 M:48.9 F did not vary from parity. Clutch sex ratio (% male) was positively correlated with clutch mean egg size, opposite to our prediction. Within clutches, however, egg size did not differ by sex. Male phalarope fitness may be more variable than previously thought, and/or differential investment in eggs may affect the within-sex fitness of males more than females. Eggs producing males were less dense than those producing females, possibly indicating they contained more yolk relative to albumen. Albumen contributes to chick structural size, while yolk supports survivorship after hatch. Sex-specific chick growth strategies may affect egg size and allocation patterns by female phalaropes and other birds.

**Keywords** Sex ratio · Parental investment · Red-necked phalarope · Reproductive strategies · Sex allocation

Communicated by I. R. Hartley

Douglas Schamel passed away during the preparation of this study.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00265-014-1800-1) contains supplementary material, which is available to authorized users.

W. B. English · D. Schamel · D. B. Lank (✉)  
Centre for Wildlife Ecology, Department of Biological Sciences,  
Simon Fraser University, Burnaby, British Columbia V5A 1S6,  
Canada  
e-mail: [dlank@sfu.ca](mailto:dlank@sfu.ca)

D. Schamel  
Department of Biology and Wildlife, University of Alaska Fairbanks,  
Fairbanks, AK 99775, USA

D. M. Tracy  
PO Box 1443, Anchor Point, AK 99556, USA

D. F. Westneat  
Center for Ecology, Evolution, and Behavior and Department of  
Biology, University of Kentucky, 101 Morgan Building, Lexington,  
KY 40506-0225, USA

## Introduction

The widespread occurrence of equal production of males and females in diploids has been explained in the context of Fisher's (1930) observation that the sexes have equal expected reproductive value, and thus negative frequency-dependent selection should operate strongly to maintain parity. Despite this generality, large deviations from parity in the production of males and females do occur, primarily related to strong barriers to dispersal and local population structure (Hamilton 1967; Maynard Smith 1978; Charnov 1982; West 2009), or differences in production costs (Fisher 1930), particularly when they are non-linear (Frank 1987). In the simplest case, assuming that offspring of either sex have the same expected fitness, males and females should be produced in inverse proportion to the parents' production costs, with costs measured as opportunity or "substitution" costs (Charnov 1982). Regardless of equilibrium population sex ratios, individual

parents may differ in their expected payoffs for producing male or female offspring as a result of their own condition or environmental situation (Trivers and Willard 1973). Even when 1:1 population sex ratios (or “population investment or allocation ratios”, West 2009) are favored, individual parents can obtain greater fitness returns if they are able to make differentially competitive members of one sex, particularly a sexually selected sex (Shuster and Wade 2003), or, conversely, differentially larger numbers of one sex versus the other.

Vertebrate populations with diffuse local population structure and overlapping generations are not the most likely places to detect sex ratio strategies (Frank 1990; West 2009), and rigorous studies with negative results have been published (e.g., Postma et al. 2011). Nonetheless, positive cases have been reported, primarily involving individual conditional strategies of sex allocation (Komdeur et al. 1997; Clout et al. 2002 for birds; West and Sheldon 2002 for ungulates). Fewer population-specific differences in equilibria (e.g., Badyaev et al. 2002), which provide the best method for testing theory’s applicability at the species level (West 2009), have been found.

The natural history of shorebirds makes them of interest for testing predictions from sex ratio theory. Clutch size exhibits little variation, with a strong mode of four. Thus, trading off numbers of offspring against brood sex composition is not a complicating factor. Shorebird young are precocial and thus sex differences in parental care costs after hatch will be smaller than in species with altricial offspring, if present at all. Despite these common features, the group has a broad range of mating and parental care systems (Pitelka et al. 1974; Szekely and Reynolds 1995), and it has recently been proposed that adult sex ratios drive much of this variation (Liker et al. 2013). Biases in primary or secondary sex ratios should both contribute towards and adapt to population equilibria, leading to different sex allocation strategies (Pitelka et al. 1974; Szekely and Reynolds 1995). In polygynous ruffs (*Philomachus pugnax*), with female-only incubation, population sex ratios are strongly female-biased at the juvenile stage (35–40 % male, Jaatinen and Lank 2010); this may originate in large part from sex ratios at hatch. Breeding females appear to bias their sex allocation towards females, the less sexually selected sex, when in poorer body condition (Thuman et al. 2003). The socially monogamous and biparental common sandpiper (*Actitis hypoleucos*) shows a seasonal trend in clutch sex ratio, with a male bias in early broods, which was interpreted as providing the territorial sex with the advantage of hatching earlier in the season. In the spotted sandpiper (*Actitis macularia*), a facultatively polyandrous sister species where females are the territorial sex, an opposite bias towards the overproduction of females in earlier clutches was predicted, but no pattern was found (Andersson et al. 2003). Male-biased sex ratios at hatch have been reported in biparental dunlin (*Calidris alpina arctica*) and ruddy turnstones

(*Arenaria interpres*), although sample sizes were small (Reneerkens et al. 2005).

Our study investigates sex ratios in the red-necked phalarope, *Phalaropus lobatus*, and due to shared life history features, we can contrast patterns in phalaropes most directly with the species mentioned above. Female phalaropes are more brightly colored, approximately 20 % larger than males, and compete aggressively for mates (Hildén and Vuolanto 1972; Reynolds 1987; Schamel et al. 2004a, b). Although lifetime variation in reproductive success has not been quantified in this species, female phalaropes are thought to have greater variation in reproductive success than males, since some females are polyandrous and can produce multiple clutches per season, while males are limited to raising one clutch per season (Hildén and Vuolanto 1972; Schamel and Tracy 1977; Reynolds 1987; Whitfield 1990; Schamel et al. 2004a). First, if male phalaropes are less expensive to produce and have lower variation in reproductive success, the Fisher/Charnov model predicts a male bias in the hatching sex ratio. Second, conditional sex allocation predicts that females being able to allocate more resources to reproduction would benefit by allocating reproductive effort towards producing highly competitive daughters, while those less able to do so should bias towards producing males. From this framework, we analyze the relationships between measures of female reproductive investment and clutch sex ratio.

Female phalaropes do not participate in incubation or chick-rearing, therefore maternal investment in offspring is limited to and should reasonably be measurable in qualities of their eggs. Polyandrous female phalaropes produce larger eggs than monogamous females (Schamel et al. 2004a), thus egg volume may provide a proxy measure of female competitiveness and/or capability for reproductive investment. In addition to biased secondary sex ratios, several forms of sex-biased egg quality have been found in other avian species. The most obvious is sexual dimorphism in egg size, which has been reported in several species (e.g., Anderson et al. 1997; Rubolini et al. 2009). In house sparrows (*Passer domesticus*), which are somewhat sexually dimorphic with slightly larger males, Cordero et al. (2000) found that eggs containing male embryos were significantly larger than those containing females, and concluded that females were likely allocating more resources to male offspring due to higher condition-dependent reproductive variance in this sex. In most species where dimorphic eggs have been found, the male-producing eggs have been larger (e.g., Müller et al. 2005; Rubolini et al. 2009; Martyka et al. 2010). Various more subtle maternal sex allocation strategies have been reported, including seasonal patterns in sex ratio (Zijlstra et al. 1992; Andersson et al. 2003), interactions between laying sequence, sex and egg provisioning within clutches (Badyaev et al. 2002), sex biases in yolk steroid concentrations (e.g., Petrie et al. 2001; Badyaev et al. 2002; Gil 2008), and other aspects of egg provisioning (e.g.,

Young and Badyaev 2004). However, with the exception of raptors (e.g., Anderson et al. 1997), few studies of sex-biased allocation in eggs have been done in species with reversed sexual dimorphism.

Assuming that higher allocation to produce more competitive female phalaropes, with their larger size and greater potential for variation in reproductive success, could provide greater fitness returns than allocating resources to produce more competitive males, we make the following predictions: (1) the embryonic sex ratio (sex ratio of all embryos, whether successfully hatched or not) will be male-biased, based on Fisher's equal reproductive value but different cost hypothesis. (2) Embryonic sex ratios will be female-biased early in the season, assuming competitive advantages accrue differentially to earlier hatched females, opposite to the pattern seen in common sandpipers (Andersson et al. 2003). (3) Females who are able to invest more in reproduction through larger eggs, possibly due to better body condition, will invest more in females, whereas less competitive individuals will bias towards males, opposite to the pattern suggested for ruffs (Thuman et al. 2003). (4) Individual eggs producing males or females may differ in size or quality, with more costly eggs more likely to produce females.

## Materials and methods

Fieldwork was conducted during May–July of 1996–1999 at Cape Espenberg, Alaska (66° 30' N, 163° 30' W), and May–July of 2011–2012 near Nome, Alaska (64° 20' N, 164° 56' W). Both sites are located on the Seward Peninsula in western Alaska and consist of wet lowland coastal tundra. Nests were found by flushing incubating males and by following mated pairs.

On finding a nest, we floated the eggs to estimate stage of incubation, initiation date, and hatch date (Liebezeit et al. 2007). Egg length and width were measured to the nearest 0.1 mm using calipers and weighed to the nearest 0.1 g using a 10 g pesola scale. Clutches in Espenberg were usually found during laying and so fresh egg mass, which correlates strongly with calculated egg volume, was also measured. Clutches in Nome were generally found during incubation, after eggs had lost mass, and therefore measurements of egg mass were not used from this site. Egg volume was calculated using the formula  $\text{volume} = 0.44077 \times \text{length in cm} \times (\text{width in cm})^2 + 0.211$ , which accounted for 93 % of variation in actual red-necked phalarope egg volume (Väisänen et al. 1972). We calculated the density of eggs from Espenberg as mass/calculated volume; these values will also vary due to differences in egg shape. As a crude index of shape, we calculate “elongation” as egg length/egg width. At both Nome and Espenberg, complete clutches were protected with predator exclosures to maximize nest success and therefore

sample size. At Espenberg, extremely high predation levels nonetheless necessitated collecting clutches after several days of incubation and using tissue from the embryos for sex analyses. This allowed us to associate individual egg metrics with sex, but no chick measurements were collected at this site. Eggshell thickness at the middle of the egg and at both ends was measured for eggs collected at Espenberg, using calipers accurate to 0.025 mm. At Nome, we visited nests regularly during incubation and daily once the first signs of hatch were visible. Since all four eggs within a phalarope clutch normally hatch within 24 h, we could not associate individual egg measurements with a specific chick, and therefore sex, in clutches that hatched. We measured chick tarsus and culmen length to the closest 0.1 mm using calipers and mass to the closest 0.1 g using a 10 g pesola scale. One or two claws were clipped to produce a small blood sample for molecular sexing, which was collected on filter paper and later dried. Unhatched eggs were dissected and embryo tissue was used, if present, to determine chick sex and test for pre-hatch sex-biased mortality (Cichon et al. 2005; Pérez et al. 2006). As we report sex ratios of all chicks or embryos sufficiently developed to provide a tissue sample, our samples fall between the classical definitions of primary (conception) and secondary (hatching) sex ratios (Mayr 1939); therefore, we refer to our data as embryonic sex ratios.

## Adult morphometrics

At both sites, we caught adults using a variety of methods, including mist nets, walk-in traps, bow nets, and salmon dip nets strung with mist netting. Each bird was given a unique color band combination and a numbered metal band. Exposed culmen was measured to the nearest 0.1 mm using calipers, flattened wing chord was measured to the nearest 0.5 mm using a straight ruler, and mass was measured to the nearest 0.5 g using a 100 g pesola scale.

Metrics of incubating males were available from nearly all nests, but since females are only present at the nest for a short time while laying, it is difficult to determine maternity. For a subset of the Cape Espenberg data set, however, we could connect individual females with their nests, primarily through behavioral observations of their pairings with individually-marked males subsequently found on the nests (Schamel et al. 2004b).

## Molecular sexing

Different DNA sexing techniques were used for samples from the two sites. Each method was tested using blood from six known-sex adults. Females had two bands near the leading edge of the gel front, while males had one band (Griffiths et al. 1998; Kahn et al. 1998).

*Nome* Blood samples from live chicks were stored on filter paper and dried. Tissue samples from failed embryos were kept in ethanol and frozen at  $-40^{\circ}\text{C}$ . DNA was isolated from blood samples using Instagene Matrix (Bio-Rad, Hercules, CA); tissue samples were treated with proteinase K and ammonium acetate. All DNA was resuspended in TE buffer. PCR was run on both blood and tissue samples using primers 2669R and 2602F. The products of PCR were run on agarose gel, and visualized with SybrSafe (Life Technologies, Carlsbad, CA).

*Cape Espenberg* DNA was extracted using a salt extraction procedure (modified from Miller et al. 1988), and cleansed of excess protein from DNA using phenol/chloroform. PCR was run using primers 2917F and 3088R (Ellegren 1996). PCR products were run on agarose gel, stained with ethidium bromide, and photographed under UV light.

### Analyses

We express sex ratio as the percent of males in a clutch or population. We tested for deviations from a population-wide embryonic sex ratio of 0.5 and for deviations from binomial expectations for clutch sex ratios using goodness-of-fit chi-square tests, restricted to 3 and 4 egg clutches for the latter. Non-random sex allocation would be supported if the distribution of sex ratios among clutches does not fit a binomial distribution (Postma et al. 2011), although failure to find such deviations would not rule out sex allocation occurring among individuals of different qualities, e.g., if similar numbers of females skewed their clutch sex ratios in each direction. Using data from Nome, we tested whether the sex of an egg affected the likelihood that the embryo would die prior to hatch using generalized linear mixed models (PROC GLIMMIX, SAS), with clutch as a random factor and restricting the unhatched sample to those from nests where one or more other eggs hatched.

Analyses involving mean sex ratios of clutches used data from both sites, but analyses involving the sex of individual eggs were restricted to the Espenberg data, while those addressing sex-biased egg mortality and chick size were restricted to Nome. Chick size was indexed with the first principal component based on mass, tarsus length, and culmen length. The PCA index accounted for 43 % of the variation in chick measurements, with positive loadings for all variables

We assessed the effects of potential covariates of clutch sex ratio using binomial logistic regressions (events per trial structure, dependent variable = number of males/number of eggs sexed per clutch) on egg characteristics, parental male and female morphometrics, and annual and seasonal (initiation date) effects. For multiple regression analyses, we report likelihood ratio  $\chi^2$  for full model effects and Wald  $\chi^2$  for partial effects.

Individual sex and egg attributes were available from Cape Espenberg, and individual sex and chick data from Nome. We present mean and standard error values for male and female egg and chick metrics. We tested for sex differences in egg and chick metrics using generalized linear mixed models (PROC GLIMMIX) with a binomial distribution, a logit link, and clutch as a random factor. With subsets of data from Espenberg, we assessed whether male and female eggs differed in shell thickness and tested whether offspring sex or egg shape were related to laying order.

All analyses were done in SAS version 9.3 (SAS Institute 2010). The primary data used in this analysis are available online as Appendix 1.

### Results

*Population embryonic sex ratios* We found no differences in annual population embryonic sex ratios, and therefore have pooled years within sites. Sex ratios, measured at both the individual egg level, or as the means of clutch sex ratios, did not differ from parity at either site or in the pooled data set (pooled: egg level 51.1 % male  $\chi^2=0.11$ ,  $df=1$ ,  $p=0.73$  clutch level 50.9 %,  $SE=2.1$  %; Table 1). The distributions of clutch sex ratios did not differ significantly from those expected under a binomial distribution (pooled samples, goodness-of-fit chi-square: three-egg clutches  $n=46$ ,  $\chi^2=2.2$ ,  $df=3$ ,  $p=0.53$ ; four-egg clutches  $n=99$ ,  $\chi^2=4.5$ ,  $df=4$ ,  $p=0.34$ ). There was a marginally non-significant difference in sex ratio between successfully hatched eggs and embryo mortalities, (unhatched 25 % male,  $n=16$ , hatched 52.6 % male,  $n=251$ ;  $F_{1,185}=3.56$ ,  $p=0.06$ ), but the sample of unhatched eggs was small.

*Clutch sex ratios* We found no evidence of seasonal or annual effects on clutch sex ratios. In a two-variable model ( $n=162$  clutches, 566 eggs), neither nest initiation date (Wald  $\chi^2=0.42$ ,  $df=1$ ,  $p=0.52$ ) nor year (Wald  $\chi^2=5.8$ ,  $df=5$ ,  $p=0.32$ , no significant interaction, Wald  $\chi^2=2.2$ ,  $df=5$ ,  $p=0.32$ ,  $p=0.82$ ) predicted clutch sex ratio. Clutch sex ratio was not associated with the mother's or father's size (three-variable models of wing, culmen, and mass: females  $n=37$ , LR  $\chi^2=0.51$ ,  $df=3$ ,  $p=0.92$ ; males  $n=138$  LR  $\chi^2=3.2$ ,  $df=3$ ,  $p=0.37$ ). However, females who produced eggs of larger mean size also produced more males: clutch sex ratio varied positively with bivariate logistic regressions of mean egg width, length, and calculated egg volume, in the pooled dataset (Table 2, Fig. 1). Significant or nearly significant regressions of sex ratio on these variables occurred independently at each site: egg width and volume at Nome (width:  $n=91$ , LR  $\chi^2=3.52$ ,  $df=1$ ,  $p=0.061$ ; volume:  $n=91$ , LR  $\chi^2=3.41$ ,  $df=1$ ,  $p=0.065$ ) and egg length and volume at Espenberg (length:  $n=$



**Table 1** Site-specific and pooled embryonic sex ratios (hatched + unhatched eggs)

Population sex ratio	Nome	Espenberg	Pooled data
Eggs (% male, <i>N</i> )	50.5, 305	51.9, 264	51.1, 569
Clutch means (% male $\pm$ SE%, <i>N</i> )	49.8 $\pm$ 3.0 %, 93	52.3 $\pm$ 2.29 %, 70	50.9 $\pm$ 2.1 %, 163

70, LR  $\chi^2=3.90$ ,  $df=1$ ,  $p=0.048$ ; volume:  $n=70$ , LR  $\chi^2=3.06$ ,  $df=1$ ,  $p=0.038$ ). This relationship between sex ratio and egg size was independent of initiation date. Egg size increased slightly with later laying dates (egg volume: slope= $+0.006$  cm<sup>3</sup>/day, being ca. 0.18 cm<sup>3</sup> or about 3 % larger, at the end of the ca. 30-day season than at the start; mixed model, clutch as random factor, no year or interaction effects, initiation date: num  $df=1$ , 768,  $F=4.43$ ,  $p=0.04$ ) years. A model of sex ratio as a function of initiation date, year, and egg volume confirmed a positive effect of egg volume, but no effect on season or year (volume: Wald  $\chi^2=6.33$ ,  $df=1$ ,  $p=0.02$ ; initiation date: Wald  $\chi^2=1.95$ ,  $df=1$ ,  $p=0.16$ ; year: Wald  $\chi^2=6.15$ ,  $df=5$ ,  $p=0.29$ ).

*Egg and chick level analyses* Sex-specific descriptive statistics for eggs and chicks of known sex are presented in Table 3. Despite the general relationship between mean egg size and sex ratio, we did not detect a difference in the volumes of eggs containing males or females within clutches (logistic regression with clutch identity as random factor:  $F_{(1,191)}=1.80$ ,  $p=0.18$ ). We also used logistic regression to model clutch sex ratios as a function of individual egg volume, egg sex, and their interaction. Egg volume again predicted clutch sex ratio (Wald  $\chi^2=5.4$ ,  $df=1$ ,  $p=0.02$ ), but there was no significant effect of volume on individual egg sex (Wald  $\chi^2=0.1$ ,  $df=1$ ,  $p=0.77$ ), nor a significant interaction, indicating that eggs of both sexes increased in size similarly with sex ratio (Wald  $\chi^2=0.5$ , 1  $df$ ,  $p=0.47$ ). The positive relationships between clutch sex ratios and egg size thus derive primarily from differences in the general size of eggs laid by each female and her sex ratio, rather than the different sizes of male and female eggs within clutches.

There were no size differences between male and female chicks, as the principal component of chick size was not a predictor of chick sex ( $F_{1,162}=0.35$ ,  $p=0.56$ ). In the subset of clutches where laying order was known, we found no relationship between order and egg sex (estimate= $-0.01$ ,  $F_{1,91}=0.01$ ,  $p=0.95$ ).

*Egg metrics* Controlling for mass, female eggs were smaller in calculated volume and male eggs larger, and controlling for volume, female eggs were heavier and male eggs lighter (egg volume  $F_{1,171}=7.0$ ,  $p=0.009$ , egg mass  $F_{1,171}=5.8$ ,  $p=0.02$ ; Table 3). There were no sex differences in egg shell thickness ( $F_{1,104}=0.01$ ,  $p=0.92$ ) that could contribute to this pattern. Eggs did not differ statistically in any size metric with respect to laying order (Table 4).

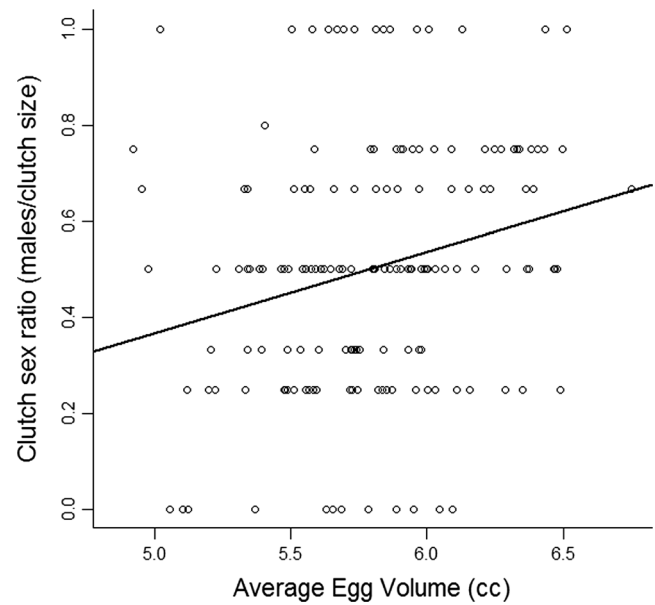
## Discussion

We tested for biases in population embryonic sex ratio, relationships between clutch sex ratio and maternal investment, and sex differences in egg metrics in the red-necked phalarope, a shorebird with a polyandrous sex-role reversed mating system, breeding at two sites over 6 years. We predicted an overall male-biased sex ratio and female-biased sex ratios in the clutches of females investing more in reproduction. We found no evidence for a substantial bias in population embryonic sex ratio or non-random distributions of sex ratios among clutches. We found no relationship between clutch sex ratio and initiation date within a season, between sites or years. Opposite to our prediction that females investing more in reproduction would bias towards females, or that females would hatch from larger eggs, clutch sex ratio (proportion of males) increased, rather than decreased, with average egg size in the clutch (Fig. 1). Females that laid larger eggs produced more males, while females laying smaller eggs produced female biases, at both sites. This pattern could occur if females made larger female-producing eggs when they produced fewer females and made smaller female-producing eggs when producing more females. However, there was no sex difference in egg size within clutches, and the slope of the relationship between egg size and sex ratio was the same for male and female eggs, with no significant difference in elevation. We thus have no evidence that individual females bias their egg size by sex. However, we did find that clutch average egg density decreased with increasing proportion of males, and individual eggs producing males appear to be less dense, which we interpret as implying a higher ratio of yolk to albumen (see below).

We found a marginally non-significant trend ( $p=0.06$ ) towards a sex bias in pre-hatch mortality. Of 16 embryo mortalities, 12 were female and 4 were male. Although the small sample size limits the strength of conclusions we can draw, our results are consistent with higher embryonic mortality in males in species where males are larger and/or more ornamented (Whittingham and Dunn 2001; Rutkowska and Cichoń 2002; Cichon et al. 2005; Pérez et al. 2006). As yet, no reason has been discovered for this disparity, but it has been suggested that if one sex is more costly to produce, that sex should suffer higher mortality, especially if resources are limited. If a female mortality bias is real, hatching sex ratios would be somewhat higher than our embryonic sex ratios (e.g., Nome embryonic sex ratio=50.5 % male,  $n=305$ ,

**Table 2** Bivariate logistic regressions of clutch sex ratio on clutch mean egg variables, using events/trial structure. Egg volume is calculated from an equation involving length and width (see “Materials and methods”),  $Df=1$  for all analyses. Significant results are in italics. *n/a* = data not available

Variable	Nome			Espenberg			Pooled					
	Odds ratio estimate (95 CI)	Likelihood ratio $\chi^2$	<i>p</i>	<i>n</i>	Odds ratio estimate (95 CI)	Likelihood ratio $\chi^2$	<i>p</i>	<i>n</i>	Odds ratio estimate (95 CI)	Likelihood ratio $\chi^2$	<i>p</i>	<i>n</i>
Width (mm)	1.6 (1.0–2.7)	3.52	0.061	91	1.34 (0.80–2.25)	1.21	0.27	70	1.48 (1.0–2.1)	4.44	0.035	161
Length (mm)	1.1 (0.9–1.4)	0.95	0.33	91	1.35 (1.0–1.8)	3.90	0.048	70	1.22 (1.0–1.5)	4.44	0.035	161
Volume (cc)	1.87 (0.96–3.64)	3.41	0.065	91	1.93 (0.92–4.06)	3.06	0.080	70	1.92 (1.17–3.14)	6.76	0.010	161
Mass (g)	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	1.3 (0.7–2.4)	0.60	0.44	63	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>
Density (mg/cc)	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	0.99 (0.98–1.00)	4.28	0.038	63	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>



**Fig. 1** Relationship between average egg volume per clutch and clutch sex ratio. As average volume increases, clutch sex ratio becomes significantly more male-biased. For illustrative purposes, we show a linear regression with slope=0.17 proportion of males per cc of egg volume, ( $n=161$ ,  $r^2=0.05$ ,  $F=8.09$ ,  $p=0.005$ )

hatching sex ratio=52.6 % male,  $n=251$ ), but any such bias would not be due to sex allocation by females, if this is defined as processes affecting the primary sex ratio.

#### Population sex ratio

We found no support for the predicted overall male bias in embryonic sex ratio. Two classes of explanations may account for this. First, sex ratio theory may be insufficiently developed to apply to this system as we predicted. Parental investment in phalaropes occurs in two stages: first, during egg-laying by the female, and second, during incubation and chick-rearing by the male. Our egg measures only capture female investment, and complementary strategies by males could theoretically alter our predictions. Whether males could or would invest differently by sex during incubation and parental care was not addressed.

Alternatively, our assumptions about the costs of biased sex allocation may need reexamination. The most general possibility is that the temporal or energetic costs of biasing sex ratio, which are currently unknown, outweigh any potential benefit. Despite robust findings of skewed sex ratios in some avian species, the mechanism responsible for this process is not understood. One proposal is that females reabsorb ova that are not the desired sex (Emlen 1997), resulting in energetic and timing costs (Pike and Petrie 2003). In the short arctic summer, female phalaropes that produce their first clutch quickly may be more likely to find a second mate. If skewing the sex ratio of their first clutch requires extra time and energy, benefits from adjusting the sex ratio may be outweighed by

**Table 3** Sex-specific metrics of individual eggs from Cape Espenberg and chicks from Nome pooled across clutches. Egg volume is calculated from an equation involving length and width (see “Materials and methods”)

Variable	Females			Males				
	Mean	95 % CL		<i>N</i>	Mean	95 % CL		<i>N</i>
Eggs								
Length (mm)	2.94	2.93	2.96	126	2.96	2.95	2.97	137
Width (mm)	2.06	2.05	2.07	126	2.07	2.06	2.08	137
Shape (length/width)	1.43	1.42	1.44	126	1.43	1.42	1.44	137
Volume (cc)	5.75	5.69	5.81	126	5.81	5.75	5.87	137
Mass (g)	6.33	6.24	6.42	111	6.35	6.28	6.43	125
Density (g/cc)	1.099	1.094	1.104	111	1.092	1.087	1.096	125
Chicks								
Tarsus (mm)	18.7	18.5	19.0	113	18.9	18.7	19.1	127
Culmen (mm)	7.75	7.65	7.85	113	7.71	7.63	7.80	127
Mass (g)	4.29	4.16	4.41	112	4.29	4.19	4.39	127

the decreased likelihood of being polyandrous. However, other species of shorebirds appear to have skewed sex ratios at hatch (e.g., Andersson et al. 2003; Thuman et al. 2003; Reneerkens et al. 2005), which suggests that whatever mechanisms operate are permissive for arctic nesting shorebirds in general.

A more specific possibility is that since the sexes do not differ measurably in size at hatch, most of the development of sexual dimorphism occurs after the period of the female parental care we measured, which stopped at egg laying. An equal embryonic sex ratio may be adaptive despite the large differences in adult size and variance in reproductive success if a female's allocation has little effect on those characteristics. Finally, variance in male fitness may be greater than previously assumed, or females might face different costs when influencing variance in fitnesses within each sex (see below).

#### Seasonal and intraclutch effects

Seasonal trends in sex ratio would be adaptive if one sex benefited more from hatching earlier (or later) in the season than the other. Such trends have been found in a variety of avian taxa including raptors (Dijkstra et al. 1990), warblers

(Neto et al. 2010), and shorebirds (Szekely et al. 2004), although only in American and European kestrels has an adaptive significance been shown (Dijkstra et al. 1990; Smallwood and Smallwood 1998). In both kestrel species, the percentage of males hatched declined over the season. Males that hatched early in the season were more likely to breed the following year than males that hatched late, but this seasonal effect was not seen in females. Thus, females breeding early in the season gained an advantage by producing more males, and females breeding later in the season produced more females, as sons they produced were less likely to breed the following year.

We found no seasonal effects on sex ratio in any year or in the combined data set. As red-necked phalaropes show low natal philopatry, and there is no known way to accurately age adults, there is currently no way of knowing if hatch date affects yearling breeding likelihood or success. Apparent seasonal effects might also be observed if female age or condition affected both initiation date and sex ratio. We found no effect of female size or condition on sex ratio, although this is complicated by the laying of multiple nests by some females. In conclusion, there do not appear to be strong seasonal patterns in sex ratios of clutches laid by female red-necked phalaropes.

**Table 4** Mean and standard deviations of metrics and sex ratios of red-necked phalarope eggs of known laying order. Elongation = length/width, volume calculated from length and width (see “Materials and methods”). None of the measures varies significantly with order (metrics: ANOVA, sex ratio: logistic regression, all  $p > 0.25$ )

Metric	Egg number ( <i>n</i> )			
	1 (21)	2 (20)	3 (23)	4 (28)
Length (cm)	2.92+0.10	2.94+0.10	2.96+0.07	2.98+0.10
Width (cm)	2.05+0.06	2.08+0.06	2.08+0.05	2.07+0.05
Elongation	1.42+0.05	1.42+0.05	1.43+0.04	1.44+0.05
Volume (cc)	5.64+0.41	5.80+0.41	5.85+0.36	5.81+0.36
Density (g/cc)	1.097+0.022	1.100+0.015	1.094+0.023	1.099+0.025
Sex ratio (proportion male)	0.62	0.50	0.59	0.61

In species with asynchronous hatching, adjusting sex with laying order can affect the level of competition between chicks, which may differ greatly in size (e.g., Genovart et al. 2003; Lezalova et al. 2005). Precocial phalarope chicks hatch within 24 h of each other, likely making hatching asynchrony and the size disparity between chicks that this can create less important compared to species where chicks hatch over the course of several days and are fed by their parents. We found no relationship between laying sequence and an egg's sex, but our power to test this was limited.

#### Re-examining assumptions about fitness variance and sex allocation

We interpreted the Trivers-Willard hypothesis as predicting that females in better condition should overproduce the sex with higher variance in fitness. Given that female phalaropes with larger reproductive investment in egg production produce more males, might male phalaropes actually be the sex with higher variation in fitness? Lifetime fitness has never been quantified in this species, given the difficulty of following survivorship and breeding success in long-lived individuals who may move between breeding attempts within a season and have low breeding site fidelity. Females have been assumed to vary more in fitness, as only some individuals are polyandrous (see "Introduction"). Nonetheless, males vary in fitness in other ways. If a female lays a clutch for a second mate, one or more of these eggs can be fertilized by sperm from her first mate (Schamel et al. 2004b). Males may differ systematically in the number of offspring that hatch or survive until fledging. Differential incubation capacity, which could relate to male body size or condition, would generate further variation in male reproductive success (English 2014). Genetic evidence supports the hypothesis that male and female phalaropes have similar variances in reproductive success. Corl and Ellegren (2012) assessed the relative amount of sexual selection occurring in six species of shorebirds through measurement of the variation in the genetic diversity of uniparentally- versus biparentally-transmitted genes. The patterns of diversity in red-necked phalaropes most closely resembled those found in monogamous species, and differed from those found in more highly polygamous species. Thus, fitness may be more variable in male red-necked phalaropes than previously considered and females may benefit more from producing more competitive males.

Finally, even if intrasexual variance is lower in males than in females, it is possible that selection on sex investment at the egg stage acts more strongly through conditional male development. If the sexes develop in such a way that variance in male fitness is influenced more than variance in female fitness by differential investment in eggs, selection could act on investment in eggs. For example, variance in traits contributing to female fitness may be determined more by incubation or

post-care events that the female does not control. Thus, even if female fitness varies more, variance in egg investment could have more effect on male fitness.

#### Sex biases in egg characteristics

Male-producing eggs had lower density values than female-producing eggs, at both the clutch and individual egg levels (Tables 2 and 3), and clutch egg density decreased as egg size increased. These patterns could be driven by sex effects on egg shape and/or egg composition. Our simple index of egg shape, based on length and width, was identical for male and female eggs (Table 3), but shape could nonetheless differ in more complex ways. Egg shape can vary with laying order (e.g., Blanco et al. 2003; Rubolini et al. 2009); therefore, any sex bias with laying order could produce differences in egg shape between the sexes. Although we found no sex ratio difference with respect to egg order, our power was limited. Väisänen et al. (1972) reported that fourth eggs of 28 red-necked phalarope clutches were longer than earlier eggs; we fail to duplicate this result in our comparably-sized data set (Table 4). To our knowledge, sex biases in laying order have not been found in shorebirds, and shape differences with laying order appear to be rare (Nol et al. 1997). Lislevand et al. (2005) found that egg width, but not length, differed with laying sequence, but found no difference in size between male and female eggs in the northern lapwing. In contrast, Nol et al. (1997) found no difference in egg width or length with laying order in the semipalmated sandpiper. We suggest that a shape bias beyond length and width is unlikely to account for the density difference between male and female eggs.

An alternative explanation is that the differences in calculated egg density result from differences in the proportions of yolk versus albumen in eggs containing males or females. The density of egg components has only been well-studied in domestic fowl, where yolk is less dense than albumen (Meur and Egbers 1990; Akashi et al. 1997). If this finding extends to phalaropes, our results suggest that female-producing eggs contain relatively more albumen and male-producing eggs relatively more yolk. Whole egg density (excluding shell) can be represented by the equation  $\text{total density} = \% \text{ yolk} \times \text{yolk density} + \% \text{ albumen} \times \text{albumen density}$ . Using density estimates from Akashi et al. (1997), of albumen = 1.034 g/ml, yolk = 1.006 g/ml, a change in overall egg density of 0.001 g/cc could represent an almost 10 % change in the proportion of yolk. Directly applying these values based on chicken eggs to our sex difference in density of 0.007 g/ml produces impossibly large differences in percent yolk, but nonetheless illustrates that small differences in egg density can translate into large differences in the proportions of egg components, specifically a substantially larger proportion of albumen in female eggs. Albumen, which is used or absorbed prior to hatch, is thought to contribute to the structural size of the chick. Conversely, a



higher proportion of yolk would make chicks less vulnerable to starvation, as yolk is an energy source not only for the embryo, but also for precocial chicks. If the maternal costs and benefits for providing the components of an egg differ with the sex of the embryo, there may be different optimal amounts of each component for each sex.

Why might female phalaropes benefit from more albumen, and males from more yolk? Domestic chicken eggs with albumen experimentally removed produced smaller and lighter chicks than control eggs (Hill 1993). Larger initial structural chick size might be more important to female chicks, which achieve larger adult size, whereas male chicks may prioritize higher survivorship. Sex-role reversal would favor such allocation. Additionally, there may be sex differences in the survivorship probabilities of chicks due to the composition of the egg. In barn swallows (*Hirundo rustica*), males appear to be more affected by the amount of albumen in an egg: chicks of both sexes were smaller when hatched from eggs with albumen removed; however, only male chicks suffered from lowered immune response (Bonisoli-Alquati et al. 2008). Our results are consistent with another study of sex-based differential allocation of egg components in ring-billed gulls (*Larus delawarensis*). Chin et al. (2012) found relatively more albumen in eggs of the larger sex despite no difference in egg size. Finally, there may be sex-related differences in the constituents of each egg component, such as differences in the concentration of steroid hormones (e.g., Gil 2008). More research in this area would help to clarify this phenomenon, for example, a non-destructive method of quantifying egg composition would better allow us to determine how it varies with egg size and how it affects chick morphology and survival.

Egg density varies not only with sex ratio, but with egg size, as these two variables are positively correlated. Studies of variation in egg composition with egg size show inconsistent patterns, and few are from shorebirds. Within precocial species, larger eggs generally have disproportionately large yolks (Williams 1994); however, there are many exceptions, including the lapwing, the only shorebird for which this information has been published (Galbraith 1988). More studies have examined the effect of egg size on chick size and survival (e.g., larger whimbrel chicks had higher survival, Grant 2008), but these are not broken down by sex.

Finally, one aspect of our data is inconsistent with the hypothesis advanced above. If chick body size is prioritized by females, we should have detected this in our chick metrics, but we did not. If females are structurally larger, but males carry larger yolk reserves, their masses might be similar despite differences in body composition related to growth strategy. Closer examination of chick morphology would help determine if dimorphic differences are in fact present.

## Conclusion

In summary, although we found that the population sex ratio did not differ from parity, female phalaropes who laid larger eggs bias production towards male offspring, and those laying smaller eggs were female-biased. Eggs producing males were less dense than eggs producing females, which may indicate a difference in egg composition, with female eggs having relatively more albumen and less yolk. Our results contradict our predictions of population-wide male-biased embryonic sex ratios and biased investment in individual female offspring, as per Fisher's and the Trivers-Willard hypotheses, under the assumptions that females are more costly to produce and have greater variation in fitness. Given our results, we must reexamine the assumption that females' reproductive success is more variable than that of males and consider the adaptive benefits of male-producing eggs being larger and having greater yolk reserves.

**Acknowledgments** Comments by two anonymous reviewers substantially improved our presentation and analyses. W.B.E.'s fieldwork was supported by Alaska Department of Fish and Game non-game program, and the National Science Foundation Office of Polar Programs grants to B.K. Sandercock. Additional funds were provided by the Northern Studies Training Program (Canada). Many thanks to E. Kwon, M. Wilson, and M. Haring for field assistance and M. Hart for laboratory analyses. D.T. and D.S. thank K. Wohl and B. Andres of the US Fish and Wildlife Service, and D. Spirtes, R. Harris, L. Selig, and L. Dalle-Molle of the US National Park Service for logistical and financial support. Additional funding came from the National Science Foundation Office of Polar Programs (OPP-9812841), the National Oceanic and Atmospheric Administration-Outer Continental Shelf Environmental Assessment Program, the University of Alaska Natural Resources Fund, United States Geological Survey-Biological Resources Division, and the Frank M. Chapman Memorial Fund. D. Matkin, P. Klein, R. Gronquist, K. Oakley, L. Johnson, J. Cole, A. Swingley, C. Acevedo, Y. Zharikov, J. Schamel, and J. T. Schamel assisted with field studies; H. Mays, R. Hanschu, and T. Roush expedited the molecular sexing. D.F.W. thanks the National Science Foundation and the University of Kentucky for indirect support of this project. W.B.E., D.S., and D.B.L. were supported by the Centre for Wildlife Ecology at Simon Fraser University.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical standards** This research was conducted under permits to W.B.E., D.B.L., and D.S. in accordance with laws and ethical guidelines in Canada and the USA.

## References

- Akashi N, Kushibiki J, Dunn F (1997) Acoustic properties of egg yolk and albumen in the frequency range 20–400 MHz. *J Acoust Soc Am* 102:3774–3778
- Anderson DJ, Reeve J, Bird DM (1997) Sexually dimorphic eggs, nestling growth and sibling competition in American kestrels *Falco sparverius*. *Funct Ecol* 11:331–335

- Andersson M, Wallander J, Oring L, Akst E, Reed JM, Fleischer RC (2003) Adaptive seasonal trend in brood sex ratio: test in two sister species with contrasting breeding systems. *J Evol Biol* 16:510–515
- Badyaev A, Hill GE, Beck ML, Dervan AA, Duckworth RA, McGraw KJ, Nolan PM, Whittingham LA (2002) Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* 295:316–318
- Blanco G, Martinez-Padilla J, Serrano D, Davila JA, Vinuela J (2003) Mass provisioning to different-sex eggs within the laying sequence: consequences for adjustment of reproductive effort in a sexually dimorphic bird. *J Anim Ecol* 72:831–838
- Bonisoli-Alquati A, Martinelli R, Rubolini D, Saino N (2008) Sex-specific effects of albumen removal and nest environment manipulation on barn swallow nestlings. *Ecology* 89:2315–2324
- Charnov EL (1982) *The theory of sex allocation*. Princeton University Press, Princeton
- Chin EH, Sharp CM, Burness G (2012) Sex-biased resource allocation in ovo in a sexually size-dimorphic species. *J Avian Biol* 43:385–389
- Cichon M, Sendecka J, Gustafsson L (2005) Male-biased sex ratio among unhatched eggs in great tit *Parus major*, blue tit *P. caeruleus* and collared flycatcher *Ficedula albicollis*. *J Avian Biol* 36:386–390
- Clout MN, Elliott GP, Robertson BC (2002) Effects of supplementary feeding on the offspring sex ratio of kakapo: a dilemma for the conservation of a polygynous parrot. *Biol Conserv* 107:13–18
- Cordero PJ, Griffith SC, Aparicio JM, Parkin DT (2000) Sexual dimorphism in house sparrow eggs. *Behav Ecol Sociobiol* 48:353–357
- Corl A, Ellegren H (2012) The genomic signature of sexual selection in the genetic diversity of the sex chromosomes and autosomes. *Evolution* 66:2138–2149
- Dijkstra C, Daan S, Buker JB (1990) Adaptive seasonal variation in the sex ratio of kestrel broods. *Funct Ecol* 4:143–147
- Ellegren H (1996) First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proc R Soc Lond B* 263:1635–1641
- Emlen ST (1997) When mothers prefer daughters over sons. *Trends Evol Ecol* 12:291–292
- English W (2014) *The evolutionary ecology of reproductive traits in the red-necked phalarope*. Simon Fraser University, Burnaby, Canada, MSc thesis
- Fisher RA (1930) *Genetical theory of natural selection*. Clarendon, London
- Frank SA (1987) Individual and population sex allocation patterns. *Theor Popul Biol* 31:47–74
- Frank SA (1990) Sex allocation theory for birds and mammals. *Annu Rev Ecol Syst* 21:13–55
- Galbraith H (1988) Effects of egg size and composition on the size, quality and survival of lapwing *Vanellus vanellus* chicks. *J Zool* 214:383–398
- Genovart M, Oro D, Ruiz X, Griffiths R, Monaghan P, Nager RG (2003) Seasonal changes in brood sex composition in Audouin's gulls. *Condor* 105:783–790
- Gil D (2008) Hormones in avian eggs: physiology, ecology and behavior. *Adv Stud Behav* 38:337–398
- Grant MC (2008) Relationships between egg size, chick size at hatching, and chick survival in the whimbrel *Numenius phaeopus*. *Ibis* 133:127–133
- Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. *Mol Ecol* 7:1071–1075
- Hamilton WD (1967) Extraordinary sex ratios. *Science* 156:477–488
- Hildén OI, Vuolanto S (1972) Breeding biology of the red-necked phalarope *Phalaropus lobatus* in Finland. *Ornis Fenn* 49:57–85
- Hill WL (1993) Importance of prenatal nutrition to the development of a precocial chick. *Dev Psychobiol* 26:237–249
- Jaatinen K, Lank DB (2010) Female-based sex ratios and the proportion of cryptic male morphs of migrant juvenile ruffs (*Philomachus pugnax*) in Finland. *Ornis Fenn* 87:125–134
- Kahn NW, St John J, Quinn TW (1998) Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. *Auk* 115:1074–1078
- Komdeur J, Daan S, Tinbergen JM, Maternans C (1997) Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. *Nature* 385:522–525
- Lezalova R, Tkadlec E, Obornik M, Simek J, Honza M (2005) Should males come first? The relationship between offspring hatching order and sex in the black-headed gull *Larus ridibundus*. *J Avian Biol* 36:478–483
- Liebezeit JR, Smith PA, Lanctot RB, Schekkerman H, Tulp I et al (2007) Assessing the development of shorebird eggs using the flotation method: species-specific and generalized regression models. *Condor* 109:32
- Liker A, Freckleton RP, Szekely T (2013) The evolution of sex roles in birds is related to adult sex ratio. *Nat Commun* 4:1587
- Lislevand T, Byrkjedal I, Borge T, Sætre G-P (2005) Egg size in relation to sex of embryo, brood sex ratios and laying sequence in northern lapwings (*Vanellus vanellus*). *J Zool* 267:81–87
- Martyka R, Rutkowska J, Dybek-Karpiuk A, Cichoń M, Walasz K (2010) Sexual dimorphism of egg size in the European blackbird *Turdus merula*. *J Ornithol* 151:827–831
- Maynard Smith J (1978) *The evolution of sex*. Cambridge University Press, Cambridge
- Mayr E (1939) The sex ratio in wild birds. *Am Nat* 73:156–179
- Meuer H-J, Egbers C (1990) Changes in density and viscosity of chicken egg albumen and yolk during incubation. *J Exp Zool* 255:16–21
- Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215
- Müller W, Groothuis TGG, Eising CM, Daan S, Dijkstra C (2005) Within clutch co-variation of egg mass and sex in the black-headed gull. *J Evol Biol* 18:661–668
- Neto JM, Hansson B, Hasselquist D (2010) Sex allocation in Savi's warblers *Locustella luscinioides*: multiple factors affect seasonal trends in brood sex ratios. *Behav Ecol Sociobiol* 297–304
- Nol E, Blanken MS, Flynn L (1997) Sources of variation in clutch size, egg size and clutch completion dates of semipalmated plovers in Churchill, Manitoba. *Condor* 99:389–396
- Pérez C, Velando A, Domínguez J (2006) Parental food conditions affect sex-specific embryo mortality in the yellow-legged gull (*Larus michahellis*). *J Ornithol* 147:513–519
- Petrie M, Schwabl H, Brande-Lavridsen N, Burke T (2001) Sex differences in avian yolk hormone levels. *Nature* 412:498–499
- Pike TW, Petrie M (2003) Potential mechanisms of avian sex manipulation. *Biol Rev Camb Philos Soc* 78:553–574
- Pitelka FA, Holmes RT, Maclean SF Jr (1974) Ecology and evolution of social organization in arctic sandpipers. *Am Zool* 14:185–204
- Postma E, Heinrich F, Koller U, Sardell RJ, Reid JM, Arcese P, Keller LF (2011) Disentangling the effect of genes, the environment and chance on sex ratio variation in a wild bird population. *Proc R Soc Lond B* 278:2996–3002
- Reneerkens J, Piersma T, Jukema J, Goeij PDE, Bol A, Meltofte H (2005) Sex-ratio and body size of sandpiper chicks at Zackenberg, north-east Greenland in 2003. *Wader Study Gr Bull* 106:12–16
- Reynolds JD (1987) Mating system and nesting biology of the red-necked phalarope *Phalaropus lobatus*: what constrains polyandry? *Ibis* 129:225–242
- Rubolini D, Ambrosini R, Romano M, Caprioli M, Fasola M, Bonisoli-Alquati A, Saino N (2009) Within-clutch egg size asymmetry covaries with embryo sex in the yellow-legged gull *Larus michahellis*. *Behav Ecol Sociobiol* 63:1809–1819
- Rutkowska J, Cichoń M (2002) Maternal investment during egg laying and offspring sex: an experimental study of zebra finches. *Anim Behav* 64:817–822

- Schamel D, Tracy D (1977) Polyandry, replacement clutches, and site tenacity in the red phalarope (*Phalaropus fulicarius*) at Barrow, Alaska. *Bird-Banding* 48:314–324
- Schamel D, Tracy DM, Lank DB (2004a) Male mate choice, male availability and egg production as limitations on polyandry in the red-necked phalarope. *Anim Behav* 67:847–853
- Schamel D, Tracy DM, Lank DB, Westneat DF (2004b) Mate guarding, copulation strategies and paternity in the sex-role reversed, socially polyandrous red-necked phalarope *Phalaropus lobatus*. *Behav Ecol Sociobiol* 57:110–118
- Shuster SM, Wade MJ (2003) *Mating systems and strategies*. Princeton University Press, Princeton
- Smallwood PD, Smallwood JA (1998) Seasonal shifts in sex ratios of fledgling American kestrels (*Falco sparverius paulus*): the early bird hypothesis. *Evol Ecol* 12:839–853
- Szekely T, Cuthill IC, Yezerinac S, Griffiths R, Kis J (2004) Brood sex ratio in the Kentish plover. *Behav Ecol* 15:58–62
- Szekely T, Reynolds JD (1995) Evolutionary transitions in parental care in shorebirds. *Proc R Soc Lond B* 262:57–64
- Thuman KA, Widemo F, Griffith SC (2003) Condition-dependent sex allocation in a lek-breeding wader, the ruff (*Philomachus pugnax*). *Mol Ecol* 12:213–218
- Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90–92
- Väisänen RA, Hildén O, Soikkeli M, Vuolanto S (1972) Egg dimension variation in five wader species: the role of heredity. *Orn Fenn* 49:25–44
- West SA (2009) *Sex allocation*. Princeton University Press, Princeton
- West SA, Sheldon BC (2002) Constraints in the evolution of sex ratio adjustment. *Science* 295:1685–1688
- Whitfield DP (1990) Male choice and sperm competition as constraints on polyandry in the red-necked phalarope *Phalaropus lobatus*. *Behav Ecol Sociobiol* 27:247–254
- Whittingham LA, Dunn PO (2001) Survival of extrapair and within-pair young in tree swallows. *Behav Ecol* 12:496–500
- Williams TD (1994) Intraspecific variation in egg size and egg composition in birds—effects on offspring fitness. *Biol Rev Camb Philos Soc* 69:35–59
- Young RL, Badyaev AV (2004) Evolution of sex-biased maternal effects in birds: I. sex-specific resource allocation among simultaneously growing oocytes. *J Evol Biol* 17:1355–66
- Zijlstra M, Daan S, Bruinenberg-Rinsma J (1992) Seasonal variation in the sex ratio of marsh harrier *Circus aeruginosus* broods. *Funct Ecol* 6:553–559