The evolutionary ecology of reproductive traits in the red-necked phalarope (*Phalaropus lobatus*)

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Abstract

Species with unusual mating systems and sex role reversal are valuable for testing theories about sexual selection and the evolution of reproductive traits that have been developed using more typical species. We used the polyandrous red-necked phalarope to test predictions about biases in primary sex ratio, factors influencing uniparental incubation and the evolution of small egg size in multi-clutching shorebird species. Egg density differed with embryo sex, and despite females being larger as adults, egg size increased with male-biased clutch sex ratio. Males had higher incubation attentiveness with greater body mass and in warmer, drier weather, while early nest initiation and increased incubation load decreased attentiveness. We detected no direct effect of experimentally manipulated incubation load on behaviour, however a greater incubation load increased the probability of nest abandonment. Our results highlight the effect of environmental conditions on reproduction in this species, and the need to reevalute our predictions about the effect of polyandrous breeding systems on reproductive traits.

Keywords: *Phalaropus lobatus*, polyandry, sex allocation, sex ratio, incubation, egg size

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Chapter 1. Introduction

1.1. Background

More than 80% percent of avian species are socially monogamous, with both parents contributing to parental care (Cockburn 2006). However, the ability for either parent to incubate has allowed other mating and parental care systems to evolve in some families. Most taxa with non-monogamous mating systems have similar systems among closely related species. For example, polygyny with some male care is common in icterids (Webster 1992), polygyny with no male care is common in grouse (Wiley 1974), while promiscuous mating, including lekking, and female only care is common in hummingbirds (Bleiweiss 1997). The Charadriiformes are an exception to this pattern: polygyny, polyandry, monogamy, and more complex mating systems all occur, and parental care can be biparental or uniparental by either sex (Pitelka et al. 1974; Reynolds & Székely 1997). This variety has been attributed to characteristics that make uniparental care possible, such as precocial young that are not fed by the parents, and the ability of one parent to successfully incubate and rear a limited number of chicks (Emlen and Oring 1977)(Emlen & Oring 1977). However, this does not explain why many different breeding systems have been selected for in closely related species that are similar in size, habitat and feeding methods.

Social polyandry, where one female forms pair-bonds and lays a clutch for two or more males in a single breeding season, is found in fewer than 1% of all avian species, yet has evolved several times within the charadriiformes (Jenni 1974; Szekely & Reynolds 1995). The environmental and social forces that have resulted in selection for polyandry are not clearly understood, although several theories have been put forth. These include the “food limited female” hypothesis that females are unable to incubate after producing the clutch, the “replacement clutch” hypothesis that females who are exempt from parental care are better able to replace lost clutches and the “fluctuating food" hypothesis that polyandry allows females to take advantage of periods of food abundace by producing multiple clutches (Erckmann 1983). However, support for these theories and others has been mixed, and it remains unclear why, if theories were true, other mating strategies such as monogamy and polygyny would be found in closely related species exposed to similar conditions (Erckmann 1983).

In species with biparental care, parents are in conflict with each other as to how much care to provide. In general, individuals benefit from providing care if it increases the likelihood that their offspring survive to independence. However, costs include the time and energy spent providing care, possible increased predation risk, and the loss of remating opportunities (Székely et al. 2006). Individuals may benefit by lowering their investment if this results in a compensatory increase in parental care by their mate (Kosztolanyi et al. 2009). Abandonment by one parent, and a shift from biparental to uniparental care, would theoretically be selected for if the fitness benefits to the abandoning parent outweigh the fitness costs of not providing care (Székely et al. 2007).

Liker et al. (2013) have shown a relationship between adult sex ratio and mating system, and suggested that biased adult sex ratios can select for increased parental care in the more numerous sex and polygamy in the rarer sex. This may be due to higher remating opportunities, and greater benefits of abandoning care to the other parent for the rarer sex (Olson et al. 2008). The relationships presented by Liker et al. (2013) are strong, but the cause-effect scenarios proposed and the strength of the adult sex ratio data used remain open to discussion. While the diversity of mating and parental care systems found in shorebirds has made this group popular for testing the evolution of sexual selection and reproductive traits, much remains to be explained.

The diversity of mating and parental care systems among shorebirds makes them useful for comparative studies of the traits associated with each system. Theories about sexual selection and the evolution of reproductive traits are often formed through observations of species with "typical" mating systems and sex roles. However, species with unusual mating systems provide tests of predictions from these theories, and the assumptions that they make. For example, Hamilton (1967) used several insect species with between-kin mating to demonstrate limits to the generality of Fisher's Principle that

1:1 sex ratios are an evolutionary stable strategy (Fisher 1930). Trivers and Willard (1973) illustrated their theory of biased sex ratio with maternal condition using caribou, which are polygynous. They suggest that females in good condition should produce more sons, as males have higher variation in reproductive success in this species. Based on this theory, we would expect the opposite in a polyandrous species, as females would have higher variance in reproductive success.

Andersson et al. (2003) used species with different mating systems and sex roles to test previously established sex ratio theory by comparing seasonal primary sex biases in two sister species, the polyandrous spotted sandpiper (*Actitis macularius*) and the monogamous common sandpiper (*A. hypoleucos*). Previous studies in raptors had shown seasonal biases in sex ratio, with males being more numerous early in the season and females later (Dijkstra et al. 1990; Smallwood & Smallwood 1998). This was attributed to a sex bias in the advantages of hatching early; earlier hatch date increased the chances of reproduction for second year males, but had no effect on second year females. Andersson et al. (2003) hypothesized that sex ratios early in the season would be male biased in the common sandpiper, where the male is the territorial sex, and female biased in the spotted sandpiper where females are the territorial sex. Although early clutches were strongly male biased in the common sandpiper, no seasonal patterns in sex ratio were found in the spotted sandpiper. Theoretically, a seasonal skew in sex ratio could mechanistically be possible in the spotted sandpiper given that it is found in its sister species; thus the lack of seasonal patterns in the spotted sandpiper suggest that the theory and assumptions behind the prediction must be reexamined. In this example, testing hypotheses across species with different mating systems has raised questions about the interpretation of a reproductive trait found in a monogamous species.

1.2. Thesis outline

In this thesis, I examine reproductive traits in the polyandrous red-necked phalarope (*Phalaropus lobatus*) and apply predictions from general reproductive theories to a polyandrous system.

In Chapter 1, I test predictions based on Fisher's sex ratio theory (1930) of equal parental allocation between offspring sexes, using molecular sexing data to determine whether female phalaropes bias egg investment towards daughters, and sex ratio towards sons. In a test of the Trivers-Willard hypothesis (Trivers & Willard 1973), I test whether females are biasing their clutch sex ratios depending on their condition. I assess whether sex ratio is biased towards females early in the season, as we would expect females, as the dominant sex, to benefit more from hatching early (Dijkstra et al. 1990; Smallwood & Smallwood 1998), and whether pre-hatch mortality is biased towards females, as the larger sex (Cichon et al. 2005; Pérez et al. 2006)

In Chapter 2, I explore the effects of factors that affect the energetic requirements of incubating males, such as weather and male mass, and factors that affect the consequences of exposure to embryos, such as embryo age, on incubation behaviour. I test whether environmental factors thought to increase adult energetic requirements such as inclement weather, or intrinsic factors such as small male size, increase the amount of time males spend off the nest, or whether males take compensatory measures to maintain incubation consistency. I test whether behavioural parameters such as recess length, frequency and the total proportion of time spent off the nest are affected by different conditions. I also quantify the relative importance of environmental variation, incubation behaviour, male morphometrics and nest characteristics on variation in duration of the incubation period.

In Chapter 3, I test the hypothesis that small male size is a selective force for the evolution of small egg size in multi-clutching shorebirds. I test whether experimental increases in incubation load measurably affect the behaviour of incubating males, and whether incubation load, directly or through behavioural mechanisms, affects the possibility of nest abandonment as a potential selective mechanism for smaller egg size.

1.3. Study species

Red-necked phalaropes are small, ground-nesting shorebirds that breed in the arctic and subarctic across Asia, North America and Europe. The majority of North American breeding red-necked phalaropes are thought to winter at sea off the Pacific coast of South America; however migratory connectivity is largely unknown in this species (Rubega et al. 2000). There are three species of phalaropes, all of which show sex role reversal, wherein females are larger, more colourful and compete for mates. Most female red-necked phalaropes are monogamous, however a small percentage of females are sequentially polyandrous and lay clutches for different males during a breeding season (Reynolds 1985). The incidence of polyandry appears to vary temporally and geographically (Whitfield 1990). Although sex differences in arrival time has not been documented at Nome, females have been shown to precede males at other sites (Reynolds et al. 1986). Prior to laying and during the laying period, phalarope pairs remain close together, however after the clutch is complete the female abandons the eggs (Schamel et al. 2004). Clutches are typically four eggs, although three and two eggs clutches occur (Sandercock 1997; personal obs., this study) As in other multi-clutching shorebirds, egg size in red-necked phalaropes is small relative to female body size compared to the relative size of eggs in single-clutching species (Ross 1979; Saether et al. 1986; Liker et al. 2001; Lislevand & Thomas 2006; but see Ward 2000). Male red-necked phalaropes perform all incubation and brood care. Attentiveness during the incubation period varies with factors such as weather and clutch age, but is generally around 70-75% (Erckmann 1981; personal obs., this study). Incubation requires a minimum of approximately 17 days, but is highly variable and can last for more than 36 days (Hilden and Vuolanto 1972; Reynolds 1985; Sandercock 1997); personal obs, this study). Eggs hatch near synchronously, and chicks remain in the nest for less than 24 hours. Chicks are precocial and mobile; tarsi are nearly fully grown at hatch (Sandercock 1997). Parental care consists of brooding, leading chicks to foraging areas, and predator distractions, and lasts for approximately 3 weeks, after which the male departs. Fledglings remain on the breeding grounds for another two weeks before starting southward migration (Reynolds 1985).

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Chapter 2. 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

2.1. 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 marginal cost/benefit ratio for producing males versus females differs, sex ratio theory predicts that parents may bias production, typically resulting in 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 may be more expensive to produce, and are thought to 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 those females allocating more resources to reproduction. We measured eggs and chicks, and used molecular techniques to sex 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 significantly 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 females may be able to affect the within-sex fitness of males more than females through differential investment in eggs. Eggs producing males were less dense than those producing females, indicating they contained more yolk relative to albumen. Albumen contributes disproportionately to embryonic chick structural size, while yolk also 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

2.2. 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 favoured, 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 & 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 (Clout et al., 2002; Komdeur et al., 1997 for birds; West & 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 & 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 (*A. macularia*), a faculatively 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 coloured, approximately 20% larger than males, and compete aggressively for mates (Hilden & Vuolanto 1972; Reynolds 1987). Although lifetime variation in reproductive success has not been quantified in this species, females 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. 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 able to do so would benefit by allocating reproductive effort towards producing highly competitive daughters, while those less able to do so should bias towards producing males. Therefore it is reasonable to look for correlates between female condition and clutch sex ratio and egg composition.

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. 2006; Gil 2008) and other aspects of egg provisioning (e.g. Young & 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 in better condition, who are able to invest more in reproduction through larger eggs, 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.

2.3. Materials and methods

2.3.1. General Methods

Fieldwork was conducted during May-July of 1996-1999 at Cape Espenberg, Alaska (66^{O}30'N, 163^{O}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 gram using a 10 gram 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 volume = 0.44077*length in cm*(width in cm)^{2}+0.211, computed for red-necked phalaropes (Whitfield, 1990), and we calculated the density of eggs from Espenberg as mass/volume. 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 4 eggs within a phalarope clutch normally hatch within 24 hours, 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 gram using a 10 gram 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.

2.3.2. 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 colour 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 gram using a 100 gram 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 behavioural observations of their pairings with individually-marked males subsequently found on the nests (Schamel et al. 2004b).

2.3.3. 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°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.

2.3.4. 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 (GLIMMIX), 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 multivariate 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 tested for evidence of differential sex allocation by females using generalized linear mixed models (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, determined if offspring sex was related to laying order. Finally, we present mean and standard error values for attributes of male and female eggs and use t-tests to look for sex differences at the individual egg and chick level regardless of allocation decisions by individual females.

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.

2.4. 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 χ2=0.11, 1 df, p=0.73 clutch level: 50.9±2.1 % male, 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: 3 egg clutches: n=46, χ^2=2.2, df=3, p=0.53; 4 egg clutches n=99, χ^2=4.5, df=4, p=0.34). There was a marginally 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 χ^2=0.42, 1 df, p=0.52) nor year (Wald χ^2=5.8, 5 df, p=0.32, no significant interaction) predicted clutch sex ratio. Clutch sex ratio was not associated with female or male parental sizes (3-variable models of wing, culmen and mass: females: n=37, LR χ^2=0.51, df=3, p=0.92; males: n=138 LR χ^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 mean egg volume, width and length 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, and egg length and volume at Espenberg. 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^3/day, being ca. 0.18 cm^3 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, den df=768, F=4.43, p=0.04) years. Modeling sex ratio as a function of initiation date, year, and egg volume confirmed an effect of egg volume, but neither seasonal or annual effects (volume: Wald χ^2=6.33, 1 df, p=0.02; initiation date: Wald χ^2=1.95, 1 df, p=0.16; year: Wald χ^2=6.15, 5 df, p=0.29).

Egg and Chick Level Analyses: despite the general relationship between mean egg size and sex ratio, we did not detect a difference in the volume of eggs containing males or females within clutches (logistic regression with clutch 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 χ^2=5.4, 1 df, p=0.02), but there was no significant effect of volume on individual egg sex (Wald χ^2=0.1, 1 df, p=0.77), nor a significant interaction, indicating that eggs of both sexes increased in size similarly with sex ratio (Wald χ^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, rather than sizes of male and female eggs within clutches.

There was no difference in size 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,for measurement means, see Table 3). 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 composition: controlling for mass, female eggs were smaller in 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; for measurement means, see Table 3). The difference in density between male and female eggs was not the result of different egg shell thickness: there was no difference in shell thickness between male and female-producing eggs ($F_{1,104}$=0.01, P=0.92).

2.5. Discussion

We tested for biases in population embryonic sex ratio, conditional sex allocation, and sex differences in egg composition 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. Females that laid larger eggs biased their sex allocation towards 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 between clutches, average egg density decreased with increasing proportion of males, and that individual eggs producing males were less dense, which we interpret as implying a higher ratio of yolk to albumen.

We found a marginally significant (p = 0.06) 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 fit the pattern predicted by other studies that have found higher embryonic mortality in males in species where males are larger and/or more ornamented (Whittingham & Dunn 2001; Rutkowska & 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 costlier 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, 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 their biasing the primary sex ratio.

2.5.1. Population sex ratio

We failed to support our 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 cost assumptions may need re-examination. The most general possibility is that the temporal or energetic costs of biasing sex ratio, which are currently unknown, outweigh a 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 who can 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 the decreased likelihood of being polyandrous. However, other species of shorebirds appear to have skewed sex ratios at hatch (e.g. Thuman et al. 2003; Andersson 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).

2.5.2. 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 (Szèkely, 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 any seasonal patterns in sex ratio in red-necked phalaropes.

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 hours of each other, likely making hatching asynchony 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.

2.5.3. Sex-biased allocation and egg composition

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 who invest greater resources 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 (Schamel et al. 2004). 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. 2004). 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 occuring 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 by differential investment in eggs than variance in female fitness, selection on maternal sex allocation will act more strongly on this. 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.

Male-producing eggs were significantly less dense than female-producing eggs, at both the clutch and individual egg level (Table 2), and clutch egg density decreased as egg size increased. Since our volume calculations did not account for egg shape, any sex difference in shape, which would itself be of interest, could partially account for this result. Both egg size and sex can differ with laying order (e.g. Blanco et al. 2003; Rubolini et al. 2009), however, to our knowledge sex biases in laying order have not been seen in any 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. Since we did not find sex differences with egg order, albeit with limited power, we suggest this is unlikely to drive our results. Assuming no biases in shape, for a given volume, egg density will vary due to proportions of yolk versus albumen, as we found no difference in shell thickness between male and female eggs. Since yolk is less dense than albumen (Meuer & Egbers 1990; Akashi et al. 1997), our results suggest that female-producing eggs contain relatively more albumen, while male-producing eggs contain relatively more yolk. Whole egg density (excluding shell) can be represented by the equation Total Density = % yolk*yolk density + % albumen*albumen density. Using density estimates from Akashi et al. (albumen = 1.034 g/ml, yolk = 1.006 g/ml, 1997), 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 literature values, based on chicken eggs, to sex difference of 0.007 g/ml produces impossibly large differences, but nonetheless illustrates that small differences in density can reflect 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. Domestic chicken eggs with albumen experimentally removed produced smaller and lighter chicks than control eggs (Hill 1993). Larger structural chick size might be more important to female chicks, which achieve larger adult size. Contrary to what we then predict, we did not detect differences in chick sizes at hatch (Table 3), but our precision with these measurements was not high. We conclude that our results suggest that male producing eggs contain relatively more yolk, which is an energy source not only for the embryo, but also for precocial chicks. Larger yolks provide an extra buffer against starvation, particularly in the first few days after hatch, and this may be more important to male chicks than larger structural size.

If the maternal costs and benefits for providing the components of each egg differ with the sex of the embryo, there may be different optimal amounts of each component for each sex. This may be due to sex-related differences in the constituents of each egg component, such as differences in the concentration of steroid hormones (e.g. Gil 2008). Alternatively, there may be sex differences in the survivorship probabilities of chicks due to the composition of the egg. For example, 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. 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 the 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. We did not detect any difference between male and female chick mass, culmen or tarsus, despite dimorphism in egg composition and size. Our measures of chick size include substantial measurement error, however, and closer examination of chick morphology would help determine if dimorphic differences are in fact present. 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.

2.5.4. 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. This difference in density likely reflects a difference in egg composition, with female eggs having relatively more albumen and less yolk. This suggests that there may be different costs associated with producing male versus female eggs. Our results did not support our predictions of population-wide male biased embryonic sex ratios and biased investment in individual female offspring as per Fisher's hypothesis and the Trivers-Willard hypothesis, 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 condition dependent than that of males, and consider the adaptive benefits of male-producing eggs being larger and having greater yolk reserves.

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2.8. Figures

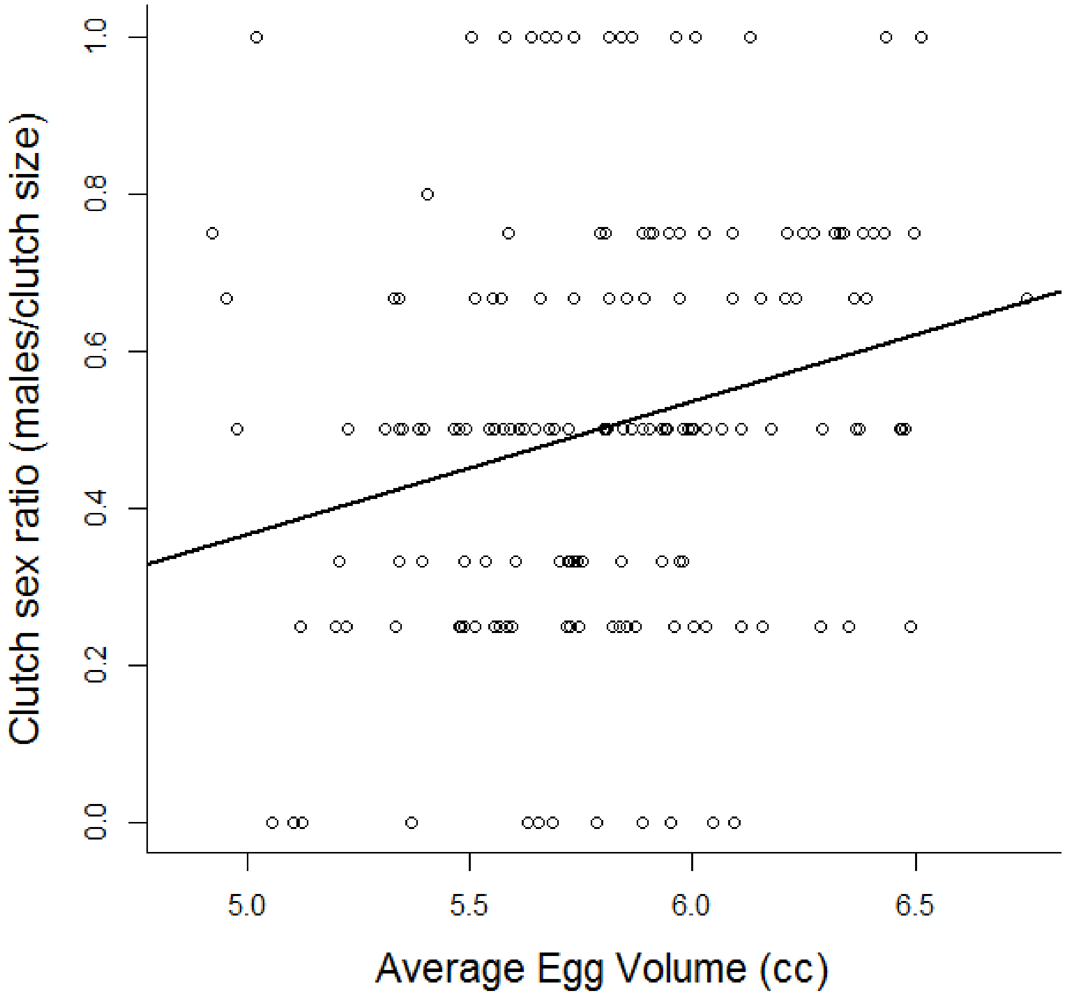

Figure 2-1. Relationship between average egg volume per clutch and clutch sex ratio. As average volume increases, clutch sex ratio becomes significantly more male biased. For illustrative purposes, we show a linear regression with slope = 0.17 proportion of males per cc of egg volume, ($n=161$, $r^2=0.05$,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

2.9. Tables

Table 2-1. Site-specific and pooled population primary sex ratio (hatched + unhatched eggs.)

Population Sex Ratio	Nome	Espenberg	Pooled Data
Eggs (% male, N)	50.5, 305	51.9, 264	51.1, 569
Clutch means (% male±SE%, N)	49.8±3.0%, 93	52.3±2.29%, 70	50.9±2.1%, 163

Table 2-2. Logistic regressions of clutch sex ratio on clutch mean egg variables, using events/trial structure. Df=1 for all analyses. Significant results are in bold. n/a = data not available.

	Nome				Espenberg				Pooled			
Variable	Odds Ratio Estimate (95 CI)	Likeli-hood ratio χ2	P	n	Odds Ratio Estimate (95 CI)	Likeli-hood ratio χ2	P	n	Odds Ratio Estimate (95 CI)	Likeli-hood ratio χ2	P	n
Egg 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
Egg 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
Egg 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
Fresh egg mass (g)	n/a	n/a	n/a	n/a	1.3 (0.7-2.4)	0.60	0.44	63	n/a	n/a	n/a	n/a
Egg density (mg/cc)	n/a	n/a	n/a	n/a	0.99 (0.98-1.00)	4.28	**0.038**	63	n/a	n/a	n/a	n/a

Table 2-3. Estimates of egg and chick variables, and results of t-tests testing for inter-sex differences. Male-containing eggs were significantly less dense than female-containing eggs

Variable	Female estimate ± SE	Male estimate ± SE	Df	T	P
Egg mass (g)	6.3 ± 0.0	6.4 ± 0.0	234	-0.34	0.74
Egg density (g/cc)	1.099 ± 0.002	1.092 ± 0.002	234	2.25	**0.025**
Egg volume (cc)	5.75 ± 0.03	5.81 ± 0.03	263	-1.35	0.18
Chick mass (g)	4.3 ± 0.1	4.3 ± 0.1	239	-0.09	0.93
Chick culmen (mm)	7.7 ± 0.1	7.7 ± 0.0	240	0.54	0.59
Chick tarsus (mm)	18.7 5± 0.1	18.9 ± 0.1	240	-1.21	0.23

Chapter 3. Incubation behaviour in the red-necked pharalope: effects of weather, incubation load and body condition

3.1. Abstract

Uniparental arctic-breeding shorebirds typically rely on exogenous resources during the incubation period, which requires them to leave the nest exposed while they forage. Inconsistent incubation can have negative effects on embryo development, however parents must balance the needs of the embryos with their own energetic requirements. Factors that affect the needs of the embryos or the parent can affect this equilibrium, resulting in changes in incubation behaviour. We measured incubation behaviour in the red-necked phalarope (*Phalaropus lobatus*), a small, polyandrous, arctic-breeding shorebird with male-only parental care. Using temperature loggers, we quantified the percent of time males spent off the nest, average recess and incubation bout length and recess frequency. We used an information theoretic approach to determine the best predictive model for each dependent variable from candidate sets including the effects of weather, initiation date, incubation load, male mass, year, instrumentation stage and length and whether the bird subsequently abandoned. We used these variables, as well as measures of incubation behaviour, to create a model set predicting total incubation length. Poor weather, high incubation loads and low male mass all resulted in more time spent off the nest. Males also spent more time off the nest early in incubation. Recess frequency was higher for birds that initiated later and those that did not abandon. Recess length increased with later initiation date and greater incubation load, and was longer in birds who subsequently abandoned. Incubation bout length was shorter in nests initiated later, and longer in nests that were later abandoned. We found no effect of incubation behaviour on incubation duration, which was longer in early nests, wetter conditions, and smaller males. By examining the factors that influence incubation behaviour and quantifying the size of the effects, we improve our understanding of the

trade-offs uniparental incubators face between their own energetic needs and the consequences of exposure to the developing embryos, and gain insight into the costs of uniparental incubation.

3.2. Introduction

Reproduction is an energetically demanding time during the annual cycle of many avian species (Williams 2012). As the time and energy that can be dedicated to each stage of reproduction is finite, parents make trade-offs in allocating their reproductive efforts, balancing the needs of their offspring with their own self maintenance and survival. In species that breed in areas where conditions are often harsh, this can present a major challenge (Martin & Wiebe 2004).

Many shorebird species make long migrations to breed in the arctic and subarctic in order to take advantage of the abundant food resources typically present during the short northern summer. Despite often sharing breeding habitat, species show tremendous variation in mating and parental care systems. Incubation and parental care can be fully biparental, performed by one sex only, or by both sexes for separate clutches (Pitelka et al. 1974; Reynolds & Székely 1997). As shorebirds typically rely on exogenous resources during the reproductive period, uniparental species must spend time off the nest to forage, leaving their eggs exposed to the arctic environment (Morrison & Hobson 2004). Eggs are generally left alone between 15-25% of the time, depending on the species and the individual (Norton 1972; Tulp and Schekkerman 2006; Reneerkens et al. 2011; Smith et al. 2012b).

While food may be plentiful during the arctic summer, it can also be highly variable, with climatic events such as high precipitation, high winds and low temperatures drastically lowering food availability while simultaneously increasing the energy required for thermoregulation and incubation (Piersma & Morrison 1994; Tulp & Schekkerman 2008; Bolduc et al. 2013). In order to maintain energy reserves during bad weather, adults must spend more time off the nest foraging (Cresswell et al. 2004; Reneerkens et al. 2011). However, as foraging is a more energetically expensive activity than incubating, in poor weather when prey items are less available it may be advantageous for incubating adults to continue incubation using stored reserves rather than expend energy foraging when returns are minimal (Piersma et al. 2003). Adults must also balance their own needs with the consequences of leaving eggs exposed during inclement weather, as prolonged exposure to suboptimal temperatures can have deleterious effects on offspring phenotype and embryo survival (DuRant et al. 2013).

Individuals can vary not only the amount of time they spend off the nest, but also the distribution of that time. Shorter, more frequent, recesses could mean that eggs do not cool to match the ambient temperature. Less variation in temperature, and less exposure to cold could be beneficial to the developing embryo (Webb 1987; Olson et al. 2006). However, longer, more infrequent recesses mean less activity around the nest, which would decrease the likelihood of predation if predators cue on the parent's movements to locate the nest (Smith et al. 2012b). Longer, less frequent recesses could also be more energetically efficient, as the cost of rewarming the eggs would be incurred less frequently (Biebach 1986). Cartar (1985) showed that white-rumped sandpipers *Calidris fuscicollis* respond to low temperature and high winds by increasing recess frequency, but not length. In contrast, Tulp & Schekkerman (2006) found that colder temperatures increased recess length and decreased frequency in three shorebird species. In general, the effects of weather on incubation behaviour have been inconsistent between studies and species. In several studies that showed little or no effects of weather, small sample size may have been a factor (eg N = 6 nests, Cartar & Montgomerie, 1987). Despite some common findings, such as increased time spent off the nest during the warmest time of the day, the effects of weather on incubation behaviour largely remain unclear (Norton 1972; Tulp & Schekkerman 2006; Reneerkens et al. 2011).

Incubating uniparental shorebirds may use fat reserves as a temporary energy source during periods of poor weather. Hegyi & Sasvari found that mass decreased in incubating female godwits (*Limosa limosa*) and lapwings (*Vanellus vanellus*) during periods of bad weather (1998). Tulp & Schekkerman (2006) found that mass decreased following periods of bad weather in uniparental little stint (*Calidris minuta)* and curlew sandpiper (*Calidris ferruginea*), while no mass change was seen in biparental dunlin (*Calidris alpina)*. This strategy may allow birds to maintain more constant incubation during poor weather despite increased energetic requirements. As part of the same study, Tulp & Schekkerman (2006) compared incubation patterns in four uniparental species (little stint, pectoral sandpiper (*Calidris melanotos*), red phalarope (*Phalaropus* *fulcarius*) and curlew sandpiper), and found a negative relationship between a species' body size and the number of recesses taken per day, with the smallest species (little stint) taking the most. This may be due to smaller species having lower proportionate energetic reserves, but it could also be affected by the unusual breeding system seen in this species: females lay a clutch for the male to incubate, then lay a clutch that they incubate themselves. As with all species where the female incubates, this makes the energetics of incubation difficult to separate from potential carryover effects from the costs of egg production.

Considerable research has been done on the effects of increased clutch size on energy expenditure by the incubating adult. In their review, Thomson et al. (1998) found that 8 of 9 studies showed increased parental energy expenditure with increased clutch size. Less work has been done on the effect of increased egg size on incubation energetic costs, a subject that is perhaps more relevant in groups such as the scolopacidae where clutch size is highly consistent (Arnold 1999). However, much of the same logic can be applied: larger eggs, like larger clutches, contain more material that requires warming and therefore may require higher energetic input. This led Lislevand & Thomas (2006) to suggest that small relative egg size in polyandrous shorebirds may be due to limited male incubation ability. Variation in the total volume of eggs being incubated may therefore have similar effects to variation in clutch size on incubation behaviour and energetic requirements.

Tulp & Schekkerman (2006) found that the frequency and length of recesses was not static throughout the duration of incubation in several uniparental shorebirds. This variance may represent changes in the benefits and consequences of each aspect of incubation behaviour due to changing conditions such as weather, the presence of predators, the energetic budget of the incubating adult and the developmental stage of the embryos. However, Tulp et al. (2009) found that variation in incubation behaviour was unrelated to stage of incubation in little stints. In contrast, Cartar & Montgomerie (1987) found that recess length decreased, and recess frequency increased as hatch approached in white-rumped sandpipers. It is generally thought that parents become more attentive as hatch approaches, potentially due to changes in the embryos' tolerance to cooling with stage of incubation (Webb 1987; Cooper & Voss 2013).

Decreased recess length as hatch approaches has been found in mountain plovers, horned larks and black-capped chickadees (Camfield & Martin 2009; Skrade & Dinsmore 2012; Cooper & Voss 2013), however other species do not show this trend (pectoral sandpiper Norton 1972; 14 species of passerine Haftorn 1988). Thus the variability between species and individuals in the relationship between stage of incubation and incubation behaviour remains largely unexplained.

Incubation behaviour, particularly long periods of parental absence, have been found to extend incubation duration, most notably in seabirds, but also in grebes, pheasants and terns (Boersma & Wheelwright 1979; Nisbet & Welton 1984; Nuechterlein & Buitron 2002; Jia et al. 2010). While the embryos of species where periodic chilling is normal are likely more resilient to such effects compared to species with normally consistent incubation, exposure to low temperatures may still slow development and even reduce growth efficiency (Webb 1987; Olson et al. 2006). If lower temperatures and incubation consistency contribute to extended incubation duration, nests would also spend longer exposed to predation (Nuechterlein & Buitron 2002).

In this study, we quantified incubation behaviour characteristics such as recess length and frequency in the red-necked phalarope (*Phalaropus lobatus*) in order to examine how incubating males are affected by weather, incubation load, body condition and seasonal effects. We then tested how incubation behaviour, together with environmental conditions, affects incubation duration. Several characteristics make the red-necked phalarope an ideal species to study the effect of weather on incubation behaviour. First, in this sequentially polyandrous species, all incubation is performed by the male, which eliminates the possible energetic effects of egg production on incubation behaviour. Second, red-necked phalaropes are among the smallest uniparental shorebirds, and thus are likely to have lower proportionate energy reserves to buffer against the effects of weather. Finally, red-necked phalaropes incubate in the arctic and subarctic, where environmental conditions can be severe and highly variable even during the summer.

In cold, windy conditions, the energetic needs of males will increase, while foraging efficiency is expected to decrease. Thus, we expect males who are exposed to poor weather to spend more time off the nest, through longer and/or more frequent recesses. Male body condition will likely affect how males are able to respond to challenging conditions, as heavier males may be in better condition or carrying more energetic reserves; thus we expect heavier birds to be buffered against the effects of weather and take fewer and/or shorter recesses. Temporal effects could result from changes in food abundance over the course of the breeding season; males nesting earlier, when less food is thought to be available, may need to spend more time off the nest foraging. Assuming that energetic requirements increase with more and/or larger eggs, we predict a positive relationship between clutch volume and time off the nest foraging; this effect will be stronger in smaller males. All of the above factors will affect total nest attentiveness; as eggs cool and stop developing when left unattended, males that spend a higher percentage of time off the nest will likely take longer to hatch their eggs.

3.3. Methods

3.3.1. Study site

Fieldwork took place in 2011 and 2012 outside of Nome, AK (64°20’N, 164°56’W) at a research site jointly run by Simon Fraser University and Kansas State University. Western sandpipers *Calidris mauri*, semipalmated sandpipers *C. pusilla* and red-necked phalaropes were studied at this site in the 1990s (e.g. Sandercock 1997). The study site is low coastal tundra with many freshwater ponds. We arrived in early May to precede the first migrants to the area and left in mid-July after all nests had hatched. Average daily temperature during the field season ranged from 0 - 15° C, although it was common to have fluctuations outside this range. Daylight is almost continuous through the summer months. Average precipitation from May-July is 3.3 cm/month, however in 2011 we had above average amounts of rain, resulting in several nests flooding; we did not categorize these as abandoned nests.

3.3.2. Field Protocol

We located nests by flushing broody males or, rarely, laying females. Phalarope nests consist of simple scrapes in the ground, with a small amount of vegetation used as lining. Eggs in complete clutches were floated to estimate the initiation date of the clutch (Liebezeit et al. 2007). Egg length and width were measured using calipers; from these measurements we calculated egg volume using a phalarope-specific equation volume = 0.44077*length in cm*(width in cm)^2+0.211 (Whitfield 1990). As nests were often found midway through incubation, and eggs lose mass over time, we did not find egg mass to be a useful measurement of egg size. Each nest was marked using a pin flag and a skewer, and the GPS coordinates were logged.

We caught males at the beginning of the season with mist-nets and hoop nets, and during incubation with nest-traps and bow-nets. Males were banded with a metal band and a unique combination of 3 colour bands. Culmen and tarsus length were measured with calipers to the closest 0.1 mm, and wing length was measured with a wing ruler to the closest 0.5 mm, following the protocols outlined in Gratto-Trevor (2004). Male mass was measured using a Pesola scale to the nearest 0.5 g.

Our weather data was measured by a weather station located on the study plot. The station took hourly measurements of air temperature, humidity, and wind speed.

Temperature logs have been used by others to measure incubation scheduling in other shorebirds including little stints (Tulp et al. 2009), red phalaropes, curlew sandpipers (Tulp & Schekkerman 2006), piping plovers (Schneider & McWilliams 2007), pectoral sandpipers (Cresswell et al. 2004) and several species of calidrid sandpipers (Norton 1972); none of these studies report negative effects of instrumentation. Schneider & McWilliams (2007) compared instrumented and uninstrumented nests, and found no difference in hatching success or predation rate. In order to minimize predation and therefore maximize the amount of incubation data gathered, we protected completed clutches using predator exclosures. These consisted of circular wire cages placed over the nest, with a mesh size that enabled adult phalaropes to easily enter and leave, but prevented larger animals such as jaegers or foxes from accessing the nest

(Schamel et al. 2004b). Once protected, no nests were depredated, and no signs of adult mortality due to the exclosures were observed.

We used two generations of data loggers: HOBO U23 and HOBO H8 (Onset, Massachusetts, USA), both of which consist of a temperature sensor probe connected to a data storage unit by a plastic enclosed wire. We covered the wire and the storage unit with vegetation to make them less visible. Sensors were inserted through the side of the nest and held in place in the middle of the clutch with a piece of plastic pushed into the ground. Temperature was measured every 30 seconds. The top of the sensor was positioned slightly lower than the top of the eggs, resulting in the tip of the probe being close to but not directly touching the brood patch. Absolute incubation temperature varied between males, likely due to slight differences in the position of the temperature probe, however male presence and absence could be easily seen as large, rapid changes in nest temperature. This system has been used successfully to measure nest attendance in red phalaropes (Tulp & Schekkerman 2006).

3.3.3. Analyses

We used Rhythm and Raven Pro 1.4 to semi-automize the process of classifying recesses (Cornell Lab of Ornithology). Rhythm converts files containing date, time and temperature data into sound files, and automatically selects incubation recesses based on selected parameters such as duration and amplitude of changes in nest temperature. We considered a drop in temperature of more than 5 degrees for more than 3 minutes to be a recess (Camfield and Martin 2009; Smith et al. 2012b). The files produced by Rhythm can be visually inspected in Raven (originally an acoustics program). We proofed the entire dataset in Raven by visually assessing the fit of recess selections made by Rhythm with the temperature recordings (Fig 3-1). Due to the cold ambient temperature in Nome, it was usually easy to see on the temperature recordings when males leave or return to their nests, however we manually made corrections when necessary. We excluded temperature recordings from the three days prior to hatch, as recesses were less clearly defined due to males changing their incubation behaviour when the eggs began to pip. As we only started recording once a full clutch was reached, we could use all of the data from the beginning of the recording. During nest checks, we occasionally found probes that had been moved from their position in the middle of the clutch, particularly when males built up their nests and buried the probes following flooding in 2011. This also occurred sporadically in both years if males moved the position of their eggs. When found, this was noted, and the temperature records examined. It was generally easy to tell when this occurred, as the maximum temperature on the records dropped substantially. In instances when this was less clear, records were discarded back to the previous nest check when the probe was known to be in the proper position. Using the recesses calculated by Rhythm, we determined the total percent of time males spent off the nest over the entire monitored incubation period as our primary variable of interest. To explore the structure of incubation in greater detail, we calculated the average length of recesses and incubation bouts, and the average number of recesses per day.

We used the behavioural measures as dependent variables with a suite of predictor variables. For each bird, we calculated the average daily low temperature and daily high wind speed for the days the nest was monitored. As these were correlated, we standardized each variable by subtracting the population sample mean and dividing by the population sample standard deviation, and subtracted wind from temperature. This created one variable for weather, with lower values corresponding to colder and/or windier conditions. We analyzed relative humidity (RH) separately from wind and temperature as these were only moderately correlated. RH is a measure of the amount of water in the air, and is related to precipitation, although the relationship is not linear (Masunaga 2012). To account for changes in incubation behaviour with incubation stage, we included the day of incubation that the nest was instrumented, as well as the duration of each nest's recording. We included male mass and wing as measures of male size, and these variables may also reflect male age, although there is currently no definitive method of aging this species (Schamel & Tracy 1988). We included total clutch egg volume, calculated as above, as a measure of incubation load. We included nest initiation date, as food availability is thought to increase throughout the season, and birds of differing quality and/or age may show differences in nest initiation date (Devries et al. 2008; Bolduc et al. 2013). We included the interaction between weather and male mass and between incubation load and male mass, as a male's condition may affect his ability to respond to challenging weather conditions or increased incubation demands.

To account for differences between years in the variables measured, as well as those not measured, such as predator abundance, we included year as a variable. As nest abandonment (defined as approximately 3 or more days without the male being seen or documented by the temperature sensors) may be the result of deficits in resources or male energy stores, which are likely to be reflected in incubation behaviour prior to abandonment, it was included as a binomial factor.

We modeled incubation length using three measures of nest attentiveness: percent off the nest, average recess length, and the average number of long recesses (>1 hour) per day. We also included the interaction between weather and the percent of time off the nest, as eggs cool more rapidly in colder, windier conditions, and thus may spend more time below the temperature at which embryonic development can occur (Feast et al. 1998; Reid et al. 2002).

To model male incubation behaviour, we created a candidate set of 24 models from the variables listed previously, including a null model and a global model (Table 3-1). We used a candidate set of 22 additional models to model incubation length (Table 3-2). We determined support for each model using an information theoretic approach by calculating Akaike's information criterion corrected for small sample size (AICc) and AICc weights (w_i) for each model. If one model had $w_i > 0.80$, we considered this model to be highly supported compared to competing models and did not model average. W_i calculated after more complex versions of the top model that increased ΔAICc by ≤ 2 for each added parameter, and did not increase log likelihood models were excluded (Arnold 2010). When no models had $w_i > 0.80$, we accounted for model selection uncertainty by model averaging over the entire set of candidate models (Burnham & Anderson 2002). As model averaging with interaction terms can create artificially large confidence intervals (Grueber et al. 2011), when interaction terms were significant we did not model average; instead, we report our top model. To facililate the interpretation of main effects appearing in significant interactions, we centered all continuous variables by subtracting the mean value from each datapoint (Schielzeth 2010). We calculated parameter likelihoods for each variable by summing the AICc weight for each model in which the variable appeared.

3.4. Results

Year differences: 2012 was significantly colder and more humid than 2011 (Table 3-3) There was no difference in male morphometrics, nest characteristics or instrumentation between years.

Percent of time off nest: The top model for average percent time off the nest included windtemp, RH, incubation load, male mass, nest instrumentation date and length as well as the male mass*incubation load and male mass*windtemp interactions, and received most of the support (AICc weight=0.72, r^2=0.34, Table 3-4). Males at nests instrumented earlier and for a longer duration spent more time off. Males that subsequently abandoned spent on average 5.5% more time off the nest compared to birds that did not abandon. As RH increased, males spent less time off the nest (parameter likelihood=0.80). The interaction between weather and male mass was moderately supported (parameter likelihood=0.77); lighter males were more affected by weather, and spent more time off the nest when exposed to poor conditions, whereas heavier males did not show a strong relationship between percent of time off the nest and weather (Fig 3-2). The effect of total egg volume also varied with male mass: males of all sizes with high incubation loads spent a similar proportion of time off the nest, however heavy males with low incubation loads spent less time off the nest (Fig 3-3). At average values of all other variables, the effect of both total incubation load and weather were positive; males spent more time off the nest as incubation load increased and as weather ameliorated. However, for lighter males, the effect of weather was negative. Similarly, for heavier males, the effect of total egg volume was positive, while for lighter males, the effect of egg volume was negative. At average values of all other variables, as male mass increased, males spent less time off the nest. Year, initiation date and male wing length were all present in the top model, however these variables has very small effect sizes and low parameter likelihoods (Tables 3-5, 3-6).

Average recesses per day: After more complex models with uninformative parameters had been removed, the model including initiation date, year, and abandonment received almost all the support (AICc weight=0.95, r^2=0.34; Table 3-4). The average number of recesses per day increased with initiation date, was higher in

2011 and in males who did not abandon their nests. No other parameters had likelihoods above 0.20.

Average recess length: The top model for average recess length included total egg volume, initiation date, instrumentation length, incubation stage when instrumented, year, and abandonment. When models with uninformative parameters were excluded, this model had an AICc weight of 0.50, and an r^2 of 0.36 (Table 3-4). The next best supported model included only initiation date, year and abandonment, and had an AICc weight of 0.20; the model with egg volume, male mass and male wing added to these parameters had an AICc weight of 0.14. All other models received little to no support (AICc weight 0-0.06). Parameter likelihoods (Table 3-5) were high for initiation date (1.00), year (1.00), and abandonment (1.00), and slightly lower for total egg volume (0.80), instrumentation length (0.74) and stage at instrumentation (0.74). Average recess length decreased with initiation date, and was higher in birds who abandoned and in 2012. Birds that were instrumented for longer took shorter recesses; as did birds that were instrumented later in incubation. Recess length also increased with total egg volume, although the model averaged 95% confidence interval for this variable, as well as for instrumentation length and stage, overlapped zero (Table 3-6).

Average incubation bout length: The top model for incubation bout length included initiation date, year and abandonment, and received an AICc weight of 0.87 when models with uninformative parameters were excluded, thus model averaging was unnecessary (Table 3-4). Incubation bout length decreased with later initiation date, and was longer in 2012 and for birds who later abandoned their nests. Although the parameter likelihoods for incubation load and instrumentation length were moderate for onbout length, we determined these to be uninformative parameters as they occurred in more complex versions of the top model that did not differ substantially from the top model in terms of log-likelihood.

Incubation length: The top model for incubation length included RH, male mass, male wing, initiation date and year, and recess length, and had an AICc weight of 0.32 and r^2=0.40 when models with uninformative parameters were excluded (Table 3-4). On average, incubation length decreased by one day for every five days later the nest was initiated. Parameter likelihoods were highest for RH (0.74), male mass (0.74) and year (0.75), however the 95% confidence intervals for male mass and year included zero. On average, incubation was longer with higher RH, decreased with increasing male mass, and was longer in 2011. Male wing length and average recess length had moderate parameter likehoods (0.55 & 0.52), however effect sizes for both these variables were small and the 95% CI included zero (Tables 3-5, 3-6).

3.5. Discussion

Different aspects of phalarope incubation behaviour appear to be affected by diverse factors, and some of these differ from those found in other uniparental arctic-nesting shorebirds. Both weather and male mass accounted for variation in the overall time spent off the nest, but we found no relationship between these variables and other measures of incubation behaviour. In contrast, initiation date and year were both important predictors of both incubation bout and recess length, but neither accounted for the total percent of time off the nest. Incubation load was a predictor of total time off and recess length. The interactions between male mass and incubation load and male mass and weather were significant for the percent of time spent off the nest; heavier males were more affected by incubation load, while lighter males were more affected by weather. Males who subsequently abandoned their nests were different from those who didn’t abandon in all aspects of incubation behaviour.

3.5.1. Predicting behaviour

Weather: Males exposed to lower temperatures and higher winds spent more time off the nest. Our results are consistent with the findings of Tulp & Schekkerman (2006) and Norton (1972) and consistent with increased energetic demands and decreased food availability during inclement weather (Piersma & Morrison 1994; Bolduc et al. 2013). In little stints, daily energy expenditure was higher during in colder weather, and birds increased the time spent off the nest (Tulp et al. 2009). This may explain why we found lighter males to be more affected by weather; they had less buffer against increased energy requirements during poor weather. In contrast, Cartar (1985) found that poor weather affected the timing of recesses in white-rumped sandpipers (*Calidris* *fuscicollis*), and Smith et al. (2012a) found that uniparental red phalaropes (*Phalaropus fulicarius*) and white-rumped sandpipers reduced time off then nest during poor weather, but were more likely to take extended (> 45 min) recesses. Smith et al. (2012a) interpreted this as birds using capital reserves to maintain incubation consistency during poor weather, but leaving to forage when reserves were depleted. These findings may reflect different strategies for coping with inclement weather between larger and smaller species uniparental incubators due to differences in the amount of reserves available. Since weather predicted total time off, it must also have affected aspects of recess length, incubation bout length or number of recesses per day, however we were unable to detect effects on separate variables, in contrast to Reneerkens et al. (2011). As weather in the arctic can change substantially during the course of a day, our daily averages of wind and temperature may have been on too coarse a scale to detect their effect on individual components of incubation behaviour. However, although Tulp & Schekkerman (2006) found that wind speed and temperature explained considerable variation in recess frequency and length in three species of uniparental arctic-nesting shorebirds, they did not find a significant effect of any weather variable on incubation behaviour in the red phalarope, despite a large sample size and fine-grained weather variables. As this species is closely related to the red-necked phalarope, and shares similar breeding and feeding habitat, this suggests that weather may have different effects on incubation behaviour in phalaropes compared to other uniparental arctic-breeding shorebirds.

In contrast to the other weather variables, males spent less time off the nest as RH increased, but we found no effect on incubation bout length, recess length or frequency. Tulp & Schekkerman (2006) found that precipitation increased recess frequency in three of four species studied, however found no effect on total time spent off the nest, measured on a per day basis. Higher RH indicates an increased likelihood of precipitation, and also increases the heat conductance of the air (Cartar & Montgomerie 1985). Although RH likely has implications for energy requirements, it may not affect food availability as much as wind and temperature, especially for a species reliant on aquatic food sources.

Nest abandonment: Males that subsequently abandoned their nests differed in incubation behaviour from males that did not. The overall percent of time spent on the nest was 5% lower in males who abandoned compared to males who did not, however as males appear to increase the amount of time they spend on the nest as incubation progresses (as seen by the negative relationship between the stage of incubation that the nest was instrumented and the percent of time off the nest), part of this difference could reflect that males who abandoned did not perform late stage incubation. However, males nearing the end of incubation took shorter, more frequent recesses, while males that abandoned took longer, less frequent recesses. Longer, less frequent recesses may be energetically less costly, as males must incur the cost of rewarming the eggs following each recess. Cresswell et al. (2004) showed that nest attendance in pectoral sandpipers is at least partially governed by adult energy resources and the cost of maintaining eggs at the required temperature. Given that male phalaropes who abandon had lower attendance and may have been employing behavioural energy saving tactics before they abandoned, this could indicate that abandonment is the result of males lacking sufficient resources to continue incubation without endangering their own survival.

Incubation load and male mass: Parameter likelihoods for incubation load were high for percent off and recess length. Males with higher incubation loads took longer recesses, which could reflect differences in cooling rates between three and four egg clutches. In great tits, Boulton & Cassey (2012) showed that smaller clutches, and eggs that are at the outside of the clutch, cool more quickly than larger clutches and less exposed eggs. While shorebirds generally show much less variation in clutch size than passerines, Norton (1970, cited in Drent 1975) found that three egg clutches cool faster than four egg clutches, potentially because the eggs in four egg clutches fit more closely together and each have less surface area exposed. If phalaropes, like passerines, time their recesses to prevent eggs from cooling below the temperature at which development stops, differences in cooling rate with clutch size could account for differences in recess length. Higher incubation loads might also be more energetically costly: uniparental male dotterels decreased attendance and lost mass when their clutches were artificially enlarged to four eggs from the normal three (Kalas & Løfaldli

1987). Male phalaropes may take longer recesses in order to forage adequately to meet the energetic demands of greater incubation loads.

Incubation load did not have a large effect on percent of time off the nest in smaller males, but in larger males percent off increased with incubation load. Heavier males spent less time off the nest in general, and especially when they had small incubation loads, suggesting that they may have the resources to increase nest attendance when the energetic demands of incubation are lower.

Males with higher masses, but not larger wing size, spent more time on the nest, however mass also affected the relationships between weather and percent off and between incubation load and percent off. The effect of weather was stronger in lighter males, while the effect of incubation load was stronger in heavier males. Fat reserves may provide a buffer against the need to increase energy intake during poor weather conditions. Tulp et al. (2002) found that little stints increased in mass with increasing breeding latitude, and interpreted this pattern as more northerly birds keeping greater reserves as “insurance” against poor weather conditions, which can be more extreme at higher latitudes. Males with higher fat stores could choose not to forage during cold periods when prey is less available, and instead forage only when conditions are best and foraging can be accomplished more quickly, resulting in less time spent off the nest.

Incubation date, stage and year: With later initiation date, males took shorter, more frequent recesses, and had shorter incubation bouts. This trend may reflect seasonal changes in other conditions; for example, increased activity around the nest has been associated with increased nest predation, therefore males may adjust their recess frequency to the predation risk (Smith et al. 2012b). Shorter, more frequent recesses should keep egg temperature more consistent, which could be advantageous for the developing embryos (Webb 1987). The cost of rewarming eggs may be higher earlier in the season, and minimized by taking fewer but longer recesses (Conway & Martin 2000).

Males at nests that were instrumented later in their incubation periods spent more time on the nest, and took shorter recesses, suggesting that attendance increases with stage of incubation. Embryos are thought to be less resistant to chilling late in incubation, which may necessitate shorter recesses. Males may also require more time to forage early in the season, due to increased energetic requirements or lower prey abundance, resulting in more time spent off the nest. Males that were instrumented for longer spent more time off the nest; this could be due to capturing behaviour from early in incubation, or could be related to incubation behaviour differences in males differing in incubation length.

Year had high parameter likelihoods for recess frequency and length, and incubation bout length. Males took fewer recesses per day in 2012, but recesses were longer, as were incubation bouts. As we did not find an effect of weather on these metrics, the significantly colder, more humid weather in 2012 may not be responsible for the effect of year. However, we experienced two major storms in 2011 that caused major flooding. Intense, concentrated weather events such as these may have large impacts on incubation behaviour, despite precipitation being lower overall in that year. Year also incorporates other variables that we did not measure, such as predator abundance or nest density, and these could also affect incubation behaviour.

3.5.2. Predicting Incubation Length

The average duration of incubation for nests found during lay was 22.3±1.5 days (range 20-24, n=10), longer than found by Hilden & Vuolanto (mean=17-18.5 days, n=20 1972) and by Reynolds (mean=18.7±2.0 days, n=47 (1985), but comparable to Kessel (mean=21.5 days n=30, 1989, cited in Sandercock 1997) and Sandercock; mean=22, n=1, Sandercock (1997). However, based on time elapsed between nest discovery and hatch, several nests found during incubation had minimum incubation durations outside this range (25 days, n=2, 26 days, n=4, 27 days, n=2, 30 days, n=1, 34 days, n=1). Incubation durations of nests where initiation date was estimated by floating the eggs also suggests that incubation duration is longer and more variable in this species than previously thought (mean=23.6±3.2, n=45). As incubation length at our site showed more variation than has been seen at other sites, we must consider whether our research activities could have contributed to extended incubation duration. However, incubation duration was strongly seasonal, and it seems unlikely that our activities (namely predator exclosures and temperature monitors) would have affected birds only early in the season in both years. Additional data from this site in 2013, when nests were monitored but did not have predator exclosures or instrumentation, supports the validity of our data, as mean incubation duration and range were similar to 2011-2012. Why incubation duration should be more variable at Nome compared to previous findings from Scandinavia and the eastern Canadian arctic is unclear, and deserves further study.

Initiation date was the strongest predictor of incubation length: for every 5 days later a nest was initiated, incubation length decreased by one day. Initiation date alone accounted for 32% of the variablility in incubation length. Although initiation date was largely uncorrelated with air temperature, we did not measure ground temperature and this likely increased throughout the season as the top layer of permafrost melted. Reid et al. (2002) found the cooling rate of eggs in artificial pectoral sandpiper nests increased with decreasing ground temperature, despite insulative nest lining material. When left exposed, lower ground temperatures may therefore cause eggs in nests laid earlier in the season to cool more rapidly below the temperatures at which development can occur. In addition, cooling due to ground temperature occurs while the male is present, and could result in lower incubation temperatures, slower development and extended incubation duration (Reneerkens et al. 2011).

Males exposed to higher humidity took longer to hatch their clutches: incubation duration increased by one day for every 4% increase in humidity. The maximum difference in humidity levels experienced by incubating males (min:66, max:89) thus represents an almost 6 day increase in incubation duration. Humidity itself may affect incubation duration through higher cooling rates due to increased heat conductance, or reflect the influence of precipitation, of which there was substantial amounts in both years (Cartar & Montgomerie 1985). Why humidity, and possibly precipitation, should have such a large effect on incubation duration, while we found little support for the effect of wind or temperature, is unclear and deserves further study. Humidity was significantly higher in 2012, although only by 2%, yet incubation duration was, on average, shorter. As there were no other significant difference between years in the variables we measured (except wind/temperature, which appears not to affect incubation duration), the difference between years was likely due to some unmeasured factor.

If males do not have sufficient reserves to ride out poor weather conditions, they may be forced to feed during poor weather, despite low foraging efficiency and increased consequences to developing embryos, while males with sufficient reserves could maintain more consistent incubation. This may be the mechanism behind the effect of male mass on incubation length: as male mass increased, incubation length decreased, although the effect size was small (on average, a mass increase of 7 g reduced incubation length by one day). Male wing length had a similar effect: larger males had shorter incubation durations, and this may reflect larger males having more energy stores.

Incubation durations were longer in males who took longer recesses; for every 17 minutes longer per average recess, incubation duration increased by one day. The average length of recesses ranged between 8 and 63 minutes, which would predict a difference in incubation length of 3.3 days. Contrary to our predictions, we did not see an effect of total percent of time off the nest, however this could be due to variation in the conditions during which the male was off the nest. For example, spending more time off the nest might have less of an effect on incubation duration if all recesses were during warm weather. For this reason, we might expect the percent of time eggs spend below a certain temperature to be a better predictor of incubation duration than the percent of time a male spends off the nest, and with different instrumentation this data might be obtainable.

3.5.3. Conclusion

In summary, incubation behaviour and duration in red-necked phalaropes is highly variable, and is affected by both environmental and intrinsic factors. Overall attendance appears to be mainly affected by weather, male mass, incubation load, and stage of incubation, however much of the variation in incubation behaviour remains to be explained. Incubation was once thought to be energetically undemanding (e.g. Walsberg & King 1978), but our work, like other recent studies, suggests otherwise. Males are faced by a trade-off between parental care and their own condition, and we believe that the large percentage of time spent off the nest suggests that meeting the demands of incubation and self-maintenance is challenging. Males who are able to do so (e.g. heavier males in good weather) increase the proportion of time spent on the nest, suggesting that eggs benefit from their doing so. Although we did not find a clear effect of nest attendance on incubation length, it seems likely based on the physiology of embryonic development this relationship should exist, and should be examined more closely.

3.6. References

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3.7. Figures

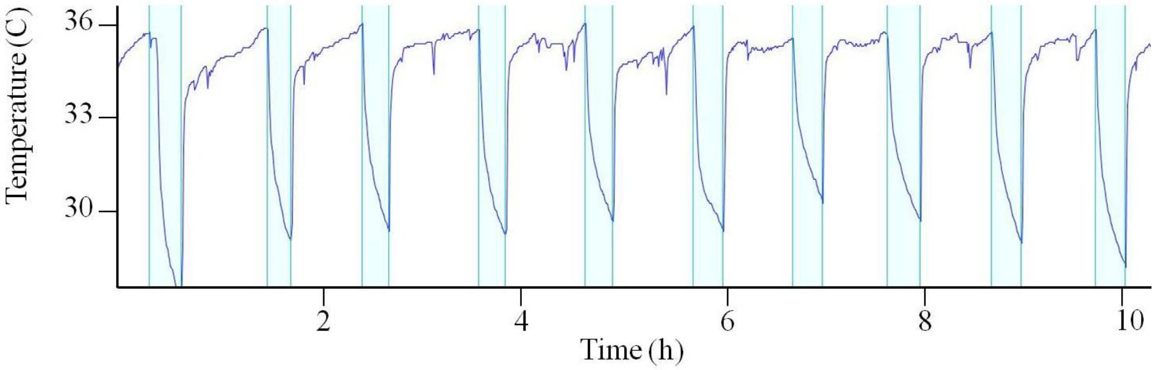
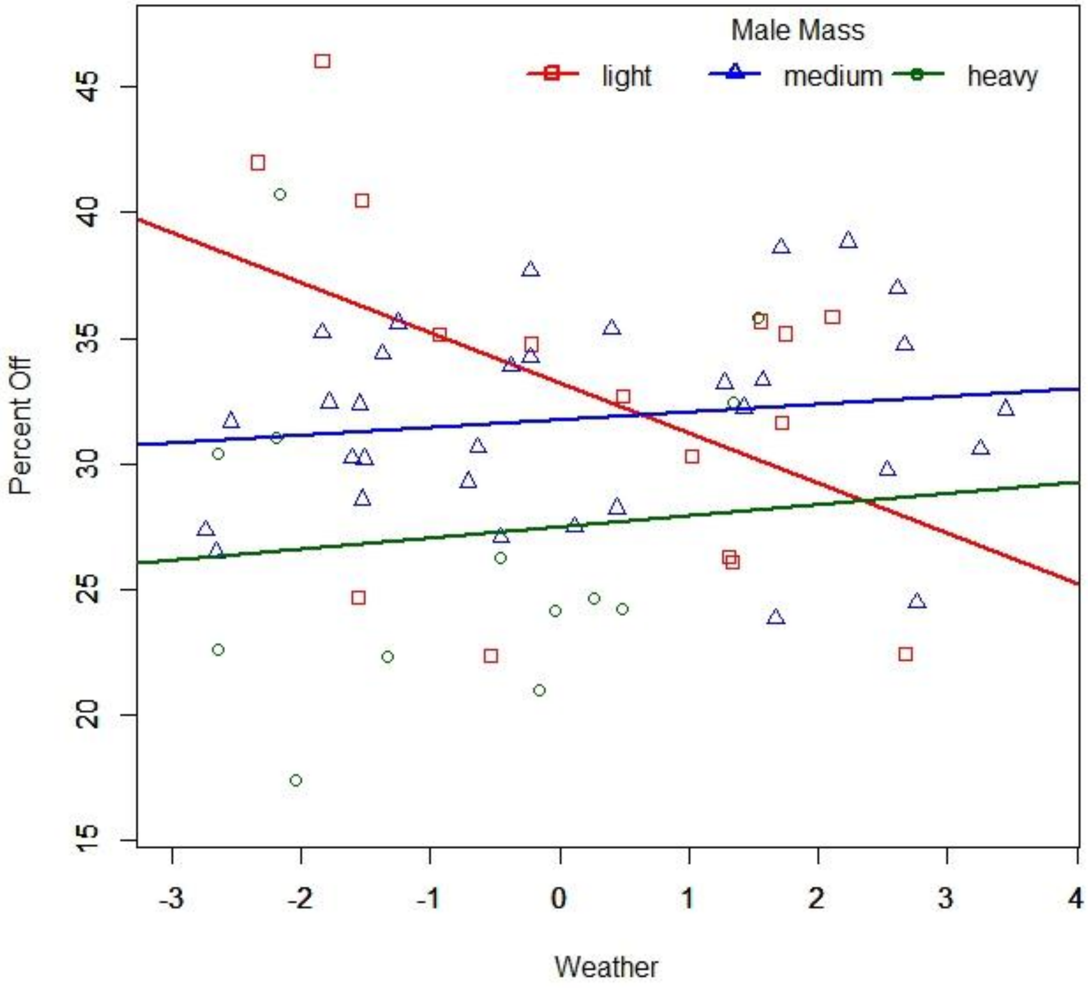
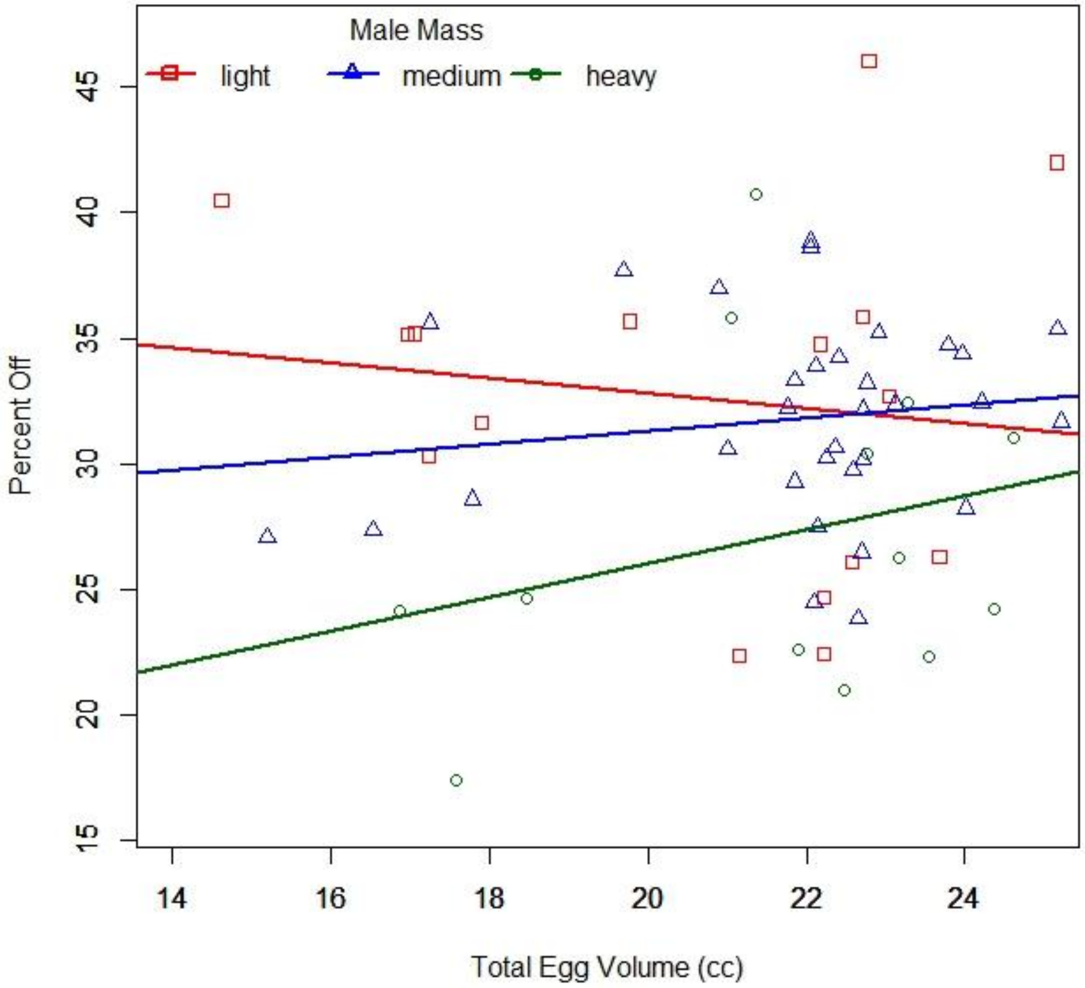


Figure 3-1. Example of temperature record visible in Raven. Recesses are visible as sudden drops in temperature. Shaded areas are recesses selected by Rhythm.



3-2. Interaction between male mass and the effect of weather on the percent of time male red-necked phalaropes spend off the nest. Small = mass≤ $\bar{x}$ - 1SD, medium = mass > $\bar{x}$ - 1SD, ≤ $\bar{x}$ +1SD, large = mass > $\bar{x}$ + 1SD. Weather variable = centered average daily low temperature – centered average high wind (lower temperatures/higher windspeed result in more negative value).



3-3. Interaction between male mass and the effect of total egg volume (incubation load) on the percent of time spent off the nest by incubating male red-necked phalaropes. Small = mass≤ $\bar{x}$ - 1SD, medium = mass > $\bar{x}$ - 1SD, ≤ $\bar{x}$ +1SD, large = mass > $\bar{x}$ + 1SD.

3.8. Tables

Table 3-1. Candidate model sets for percent off, average recesses per day, average recess length and average incubation bout length. Residual distribution approximated normal for all dependant variables, which were therefore not transformed. Windtemp” refers to average daily low temperature (centrered) – average daily high wind speed (centered). “RH” is the relative humidity. “Inc load” is the total volume of eggs being incubated. “Mass” is male mass in grams. “Wing” is male wing length in millimeters. “Init” is the date of nest initiation, in days since May 1. “Inst length” is the number of days nest attendance was monitored. “Year” is categorical with two levels, either 2011 or 2012. “Inst stage” is the day of incubation that the nest began being monitored. “Abandon” is a binary variable, whether the male later abandoned the nest.

Model
1
windtemp + xRH + toteggvol + mmass + mwing + jinit + hobolen + year + hoboininc + abandyn + mmass*windtemp + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + jinit + hobolen + year + hoboininc + abandyn + mmass*windtemp + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + mwing + jinit + abandyn + mmass*windtemp + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + hobolen + hoboininc + abandyn + mmass*windtemp + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + mwing + jinit + hobolen + year + hoboininc + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + jinit + hobolen + year + hoboininc + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + hobolen + hoboininc + mmass*toteggvol
windtemp + xRH + toteggvol + mmass + mwing + jinit + hobolen + year + hoboininc
windtemp + xRH + toteggvol + jinit + hobolen + year + hoboininc + abandyn
windtemp + xRH + toteggvol + jinit + hobolen + year + hoboininc
windtemp + xRH + jinit + hobolen + year + hoboininc
toteggvol + mmass + mwing + jinit + hobolen + year + hoboininc + abandyn + toteggvol*mmass
toteggvol + mmass + jinit + hobolen + year + hoboininc + abandyn
toteggvol + mmass + mwing + jinit + hobolen + year + hoboininc + abandyn
toteggvol + jinit + hobolen + year + hoboininc + abandyn
toteggvol + mmass + mwing + jinit + year + abandyn
toteggvol + mmass + mwing + abandyn
jinit + hobolen + year + hoboininc + abandyn
jinit + year + abandyn
windtemp + toteggvol + mmass + mmass*windtemp + mmass*toteggvol
windtemp + toteggvol + mmass + jinit
windtemp + mmass + jinit
windtemp + xRH
mmass + mwing

Table 3-2. Candidate model sets for incubation length. "Percent off" is the overall proportion of time a male spent off the nest over the entire incubation period monitored. "Xoffmin" is average recess duration, in minutes. "Xonbout" is average incubation bout length, in minutes. "Recess frequency" is the average number of recesses per day. "Xhouroff" is the average number of recesses per day that lasted more than one hour. For other variable definitions, see Table 3-1.

Model
1
percentoff + xoffmin + xhouroff + windtemp + xRH + toteggvol + mmass + mwing + jinit + year + percentoff*windtemp
percentoff + xoffmin + xhouroff + toteggvol + mmass + mwing + jinit + year
windtemp + xRH + toteggvol + mmass + mwing + jinit + year
percentoff + xoffmin + xhouroff + windtemp + xRH + mmass + mwing + jinit + year + percentoff*windtemp
percentoff + xoffmin + xhouroff + windtemp + xRH + toteggvol + jinit + year + percentoff*windtemp
percentoff + xoffmin + xhouroff + windtemp + xRH + jinit + year + percentoff*windtemp
percentoff + xoffmin + xhouroff
percentoff + windtemp + xRH + mmass + mwing + jinit + year + percentoff*windtemp
percentoff + windtemp + xRH + mmass + mwing + jinit + year
xoffmin + windtemp + xRH + mmass + mwing + jinit + year
xoffmin + xhouroff + windtemp + xRH + mmass + jinit + year
windtemp + xRH + mmass + jinit + year
percentoff + mmass + mwing + jinit + year
percentoff + xoffmin + mmass + windtemp
percentoff + windtemp + mmass
jinit + xoffmin
xoffmin + xRH + toteggvol + mmass + mwing + jinit + year
xoffmin + xRH + mmass + mwing + jinit + year
Windtemp
Percentoff
Jinit

Table 3-3. Annual means (±SD) and comparisons from two sample t-tests of weather, morphometric measurements and nest variables. Variables significantly different between years are in bold and marked with and asterisk

Variable	2011 (n=26)	2012 (n=35)	t-value	p-values
Windtemp	0.91±1.52	-0.68±1.60	3.95	**<0.001***
RH	81.4±3.4	83.6±4.9	-2.08	**0.042***
Incubation load	21.97±1.77	21.18±3.00	1.29	0.204
Male mass	32.1±3.0	33.5±2.7	-1.79	0.079
Male wing	109.7±3.1	109.5±2.7	0.359	0.722
Initiation date	34.2±8.0	35.9±7.6	-0.845	0.402
Instr length	11.3±5.4	11.7±5.8	-0.300	0.765
Instr date	12.8±5.0	10.7±4.6	1.68	0.098

3-4. Models within 4 ∆AICc of top model. AICc weight calculated after models with uninformative parameters excluded.* uninformative model – same as top model with additional parameters, ∆AICc within 2*number of additional parameters. For variable definitions, see Table 3-1.

Dependent variable	Model	K	AICc	deltaAIC	AICc w_i
Incubation length	Off length + RH + mass + wing + init + year	8	227.3133	0	0.32
	init date	3	228.1852	0.87	0.20
	Windtemp + RH + mass + init + year	7	228.3729	1.06	0.19
	Off length + RH + toteggvol + mass + wing + init + year	9	230.0532	2.73*	-
	Off length + windtemp + RH + mass + wing + init + year	9	230.2268	2.91	0.06
	Init + off length	3	230.5594	3.25	0.06
Percent off	Windtemp + RH + inc load + mass + inst length + inst stage + abandon + mass*windtemp + mass*inc load	11	376.8642	0	0.71
Average recesses per day	Init + year + abandon	5	403.6718	0	0.96
Average recess length	Inc load + init + inst length + year + inst stage + abandon	8	440.0123	0	0.573
	Inc load + mass + init + inst length + year + inst stage + abandon	9	442.1203	2.11*	-
	Init + year + abandon	5	442.1734	2.16	0.195
	Inc load + mass + wing + init + year + abandon	8	442.8149	2.80	0.141
	Windtemp + RH + incload + init + inst len + year + inst stage + abandon	10	443.9846	3.97*	-
Average incubation bout length	Init + year + abandon	5	510.10	0	0.86

3-5. Parameter likelihoods for predictors of incubation behaviour and incubation length, calculated by summing the AICc weight of all models in which the parameter appeared. Values > 0.70 are shown in bold. For variable definitions, see Table 3-1.

Y	Wind temp	RH	Inc load	Mass	Wing	Init	Inst length	Year	Inst Stage	Aband	Windtemp *Mass	Inc load *Mass
Percent off	0.80	0.80	0.93	0.92	0.11	0.26	0.99	0.26	0.99	0.97	0.77	0.89
Recesses per day	0.01	0.01	0.14	0.08	0.07	1.00	0.19	1.00	0.185	0.99	<0.01	<0.01
Average recess length	0.06	0.06	0.80	0.31	0.16	1.00	0.74	1.00	0.74	1.00	<0.01	0.01
Average incubation bout length	0.07	0.07	0.58	0.34	0.26	0.56	0.47	0.91	0.47	0.95	<0.01	0.09

	Percent off	Average recess	Long recess	Wind temp	RH	Inc load	Mass	Wing	Init	Year	Percent off*windtemp
Inc length	0.09	0.52	0.03	0.38	0.74	0.10	0.74	0.55	1.00	0.75	0.03

3-6. Model averaged parameter estimates ± unconditional SE for models where model averaging was used, best model parameter estimates ± SE where model averaging was unnecessary due to top model having AICc weight > 0.90. Variables marked with the symbol "-" for top model parameter estimates did not occur in the top model. For variable definitions, see Table 3-1.

Y	intercept	Windtemp	RH	Inc load	Mass	Wing	Init	Inst length	Year	Inst Stage	Abandon	Windtemp *mass	Inc load *mass
Model averaged estimates ± unconditional standard error													
Average offbout length	25.61 ±23.50	0.02 ±0.08	-0.02 ±0.03	0.97 ±0.48	-0.07 ±0.19	-0.06 ±0.09	-0.31 ±0.16	-0.1 ±0.20	6.94 ±2.46	-0.4 ±0.27	11.10 ±3.68	0.0002 ±0.0003	-0.0007 ± 0.002
Parameter estimates for best supported models ± standard error													
Percent off	221.89 ±65.99	-6.75 ±4.64	-0.17 ±0.17	-7.67 ±3.01	-5.72 ±2.03	-	-	0.35 ±0.14	-	-0.25 ±0.17	5.54 ±2.02	0.22 ±0.14	0.25 ±0.09
Average offbouts per day	15.74 ±3.85	-	-	-	-	-	0.30 ±0.105	-	-7.04 ±1.63	-	-6.45 ±2.27	-	-
Average onbout length	61.07 ±9.95	-	-	-	-	-	-0.66 ±0.27	-	20.86 ±4.21	-	15.03 ±5.82	-	-
Y	intercept	Percent off	Average offbout	Long offbouts	Windtemp	RH	Inc load	Mass	Wing	Init	Year	Percent off*windtemp	
Model averaged estimates ± unconditional standard error													
Inc length	31.71 ±11.94	0.005 ±0.008	0.02 ±0.02	0.001 ±0.01	0.08 ±0.15	0.18 ±0.08	-0.006 ±0.02	-0.16 ±0.10	-0.10 ±0.08	-0.22 ±0.05	-0.86 ±0.65	-0.001 ±0.001	

Chapter 4. Small eggs for small males: has male incubation ability selected for decreased egg size in the polyandrous red-necked phalarope?

4.1. Abstract

Small egg size relative to female body size in multi-clutching shorebirds with uniparental male care has long been interpreted as an adaptation for reducing the per egg cost of egg production. Alternatively, the "small male" hypothesis suggests that limitations on male incubation ability have selected for small egg size. If incubation of larger eggs is more energetically demanding, we expect males with higher incubation loads (total volume of eggs incubated) to (1) spend more time off the nest foraging and (2) be more likely to abandon their nests. We tested whether incubation load affects incubation behaviour by experimentally increasing total clutch volume in 12 red-necked phalarope (*Phalaropus lobatus*) nests, controlling for the effects of weather, stage of incubation, year, and male size. With a sample of 62 nests, we used an information theoretic approach to determine whether incubation load was included in the best model for predicting abandonment, in addition to weather, male size, and incubation behaviour. We show mixed support for the "small male" hypothesis. While nest attendance was lower in lighter males, during windy weather, and in the colder/wetter of the two years, and increased closer to hatch, we found no effect of incubation load. However, our manipulation was short and sample size small. Recess length, which we have previously shown in observational studies with larger samples sizes to increase with incubation load, was the best predictor of abandonment. For every one minute increase in average recess length, the odds of abandonment increased by 7%. Thus nest abandonment by males with larger incubation loads could be a selective mechanism for the evolution of small egg size in multi-clutching shorebirds.

4.2. Introduction

In the simplest terms, selection favours those individuals who can produce the greatest number of viable offspring and therefore pass on more of their genes to the next generation. Individuals cannot, however, produce an unlimited number of high quality offspring: there are upper limits to the time and energy allocated to reproduction. This means trade-offs must be made between various components of reproductive investment. One of the most widespread examples of this is the trade-off between offspring number and size, which can be seen within and between species across a broad variety of taxa, including teleost fish (Kamler 2005), insects (García-Barros 2000) and plants (Jakobsson & Eriksson 2000). In birds, where parental care is often extensive, the time and energy required for incubation and parental care must be factored into the equation, and clutch size in some species is thought to be limited by particular stages (Lack 1946; Monaghan & Nager 1997). However, in avian species where incubation and parental care are not rate-limiting stages, we may find that females face a trade-off between egg number and size.

Egg size is highly variable among females, and this variation has long been thought to reflect on chick phenotype and quality. However, after accounting for parental effects, many studies testing the effect of egg size on offspring quality have not shown significant results, especially in species with altricial young (Williams 2012). Support for a relationship between egg size and offspring quality appears stronger in species with precocial chicks, where experimental studies accounting for parental effects have supported larger egg size being linked to chicks with larger body size, higher survival, improved homeothermic capabilities, higher growth rates, and an increased likelihood of recruiting into the population (Dawson & Clark, 1996, 2000; Göth & Evans, 2004; Pelayo & Clark, 2003; Rhymer, 1988; Whitehead et al., 1990; reviewed in Williams 1994; Krist 2010; Williams 2012). Higher survival rates have largely been attributed to greater energetic reserves, however other traits related to larger size, such as thermoregulatory ability, may play a role (Rhymer 1988). Although most studies on precocial birds have focussed on waterfowl, a positive effect of egg size on offspring quality has been found in multiple species of shorebirds: both Galbraith (1988) and Blomqvist et al. (1997) showed that in lapwings (*Vanellus vanellus*), larger eggs produce larger chicks, which have higher survival rates than smaller chicks. Similarly, Amat et al. (2001) found that in Kentish plovers (*Charadrius alexandrinus*), larger eggs produced larger chicks, and within a clutch, heavier chicks were more likely to recruit into the breeding population. In whimbrel (*Numenius phaeopus*), larger eggs produced larger chicks with higher survival, both within and between clutches (Grant 2008). No study has found an advantage to smaller chick size, although many studies fail to show any directional patterns (see Williams 1994), and stabilizing selection on egg size, through tradeoffs against egg number and other components of parental fitness, must be widespread.

Shorebirds are generally thought of as having relatively invariant clutch sizes, which would limit the trade-off between egg size and clutch size, (although see Sandercock et al. 1999). However, in some multi-clutching species females regularly lay two or more clutches during a breeding season. These can be loosely divided into two main groups: polyandrous species, where females lay clutches for several males and perform no parental care (e.g. jacanas, phalaropes) and species where females will lay a clutch for a male to incubate and another that she may incubate herself (e.g. sanderlings, Temminck's stints). Species in the latter group can be either monogamous (both clutches fertilized by the same male) or polyandrous (female mates with a different male prior to laying the clutch she will incubate). Despite the positive relationship between egg size and offspring survival, multi-clutching shorebirds produce eggs that are smaller relative to female body size than those of single-clutching species, a pattern highlighted by Ross (1979). Two main hypotheses have been proposed to explain this pattern: (1) small eggs are the result of an energetic trade-off by females between egg size and egg number (Ross 1979; Saether et al. 1986; Liker et al. 2001), and (2) eggs are sized to match the body size of incubating males, which are often smaller relative to female size in multiclutching compared to single-clutching species (Saether et al. 1986; Liker et al. 2001; Lislevand & Thomas 2006).

Based on a comparative analysis, Ross (1979) concluded that small eggs are an adaptation to decrease the amount of resources females allocate to each egg, reducing the additional resources required to lay multiple clutches. In addition, Ross' analyses suggested that female multi-clutching shorebirds have increased in body size over the course of their evolution, perhaps to decrease the relative cost of each egg. Saether et al.'s 1986 study confirmed that eggs are relatively smaller in multi-clutching shorebirds, including polyandrous species, and considered the effect of egg size on incubation length. Both studies concluded that tradeoffs against numbers of eggs potentially to be laid annually, mediated by female energetics, favour the production of smaller relative egg size in multi-clutching shorebirds. Additional support was provided by Liker et al. (2001), who expanded on Ross' work by accounting for phylogentic non-independence, increasing the number of species and including clutch size as a variable. Liker et al. (2001) found no relationship between male body size and egg size residuals from female body size, which they felt eliminated the small male hypothesis. They concluded that the decrease in egg size in multi-clutching species is a mechanism for reducing cost per egg.

If females face a trade-off between egg size and number, a total limit on allocation is implied. However, a number of studies have cast doubt on whether egg production by female multi-clutching shorebirds is actually limited by energetic and nutritional requirements. Although food availability appeared to determine the onset of laying in polyandrous spotted sandpipers, egg-removal experiments during the laying period by Lank *et al.* (1985) showed that female are capable of laying more eggs than they normally do, which suggests that the ability to produce and provision eggs is not limiting. Lank *et al*. found no evidence of correlation between food levels and and either egg size or laying interval, which again suggests that female egg production is not nutrient limited in that system. Other shorebirds are capable of laying up to five nests in a breeding season, suggesting that the costs producing eggs need not limit reproductive output (Amat *et al.* 1999; Reed *et al.* 1997). Together, these studies suggest that female multi-clutching shorebirds are not generally nutritionally limited, and therefore may not need to make a trade-off between egg size and number.

If the function of smaller eggs is not to allow the female to produce more eggs, why would they have evolved? Lislevand & Thomas (2006) showed that in multi-clutching shorebirds where the male incubates and is smaller than the female, egg size decreases relative to female body size as female-biased size dimorphism increases. Thus egg size is more closely matched to male body size than female body size, as expected under the small male hypothesis. By what mechanism could male incubation ability select for smaller egg size? If males are unable to cover larger eggs due to the size of their brood patch, eggs might be inadequately incubated and embryo development might suffer (Lislevand & Thomas 2006). If females size eggs to fit under males, this could explain why Temminck's stints lay smaller eggs in the first clutch, which is incubated by the smaller male, and larger egg in the second nest, which is incubated by the larger female (Lank, unpublished). Between species, smaller eggs take less time to hatch, and Saether et al. (1986) suggested that decreased incubation time would lower the cost of incubation for uniparental males, allowing them to perform more extensive parental care after hatch. Either of these mechanisms could result in selection for smaller eggs due to male incubation ability.

The red-necked phalarope (*Phalaropus lobatus*) was included in the analyses of Ross, Saether et al., Liker et al. and Lislevand & Thomas. Polyandrous females phalaropes lay two or more clutches in a season and egg size is small compared to female body size (Schamel et al. 2004b). Despite the relatively small size of phalarope eggs, they still represent a substantial proportion of the females body weight, especially when multiple clutches are produced (Schamel et al. 2004a). Females phalaropes must acquire the nutrients required for egg production on the breeding grounds whilst attempting to court and defend potential mates (Klaassen et al. 2001). These activities are largely mutually exclusive, thus a female must allocate time between feeding and courting. Smaller eggs require less energetic input, and would allow the female to allocate more time to finding a mate as less time would have to be spent acquiring resources. Additionally, small egg size might reduce the demands of incubation on males. If lower incubation loads are less energetically costly to incubate, smaller eggs could allow males to spend more time on the nest and improve incubation consistency.

Incubation is an energetically costly stage of reproduction for uniparental shorebirds (Piersma et al. 2003). If the risks to survival and future reproductive opportunities are higher than the potential fitness benefits of continuing incubation, males would benefit from abandoning their clutch. If incubation load increases the burden of incubation, males with larger incubation loads may abandon at a higher frequency. As abandonment is relatively common in this species (14 %, Reynolds 1985; 15 %, this study), it has the potential to be a selective force on egg size, as females laying larger eggs could suffer lower fitness despite large eggs being beneficial, all else being equal.

We expand on the “small male” hypothesis by suggesting that male incubation ability has selected for smaller eggs not only to improve fit against the brood patch, but also to reduce the energetic burden of incubation. We test this in red-necked phalaropes two ways: (1) by measuring changes in incubation behaviour in response to experimentally increased incubation loads through the addition of eggs or egg swaps with larger eggs and (2) by testing whether nest abandonment can be predicted by incubation load, or by incubation behaviours that are affected by incubation load (Chapter 2). We predict that males with increased incubation loads will spend more time off the nest in order to forage adequately to meet increased energetic demands, and that males who have higher incubation loads will be more likely to abandon .

4.3. Methods

4.3.1. Study site

Fieldwork took place in 2011 and 2012 outside of Nome, AK (64°20’N, 164°56’W) at a research site jointly run by Simon Fraser University and Kansas State University. Western sandpipers *Calidris mauri*, semipalmated sandpipers *C. pusilla* and red-necked phalaropes were studied at this site in the 1990s (e.g. Sandercock 1997). The study site is low coastal tundra with many freshwater ponds. We arrived in early May to precede the first migrants to the area and left in mid-July after all nests had hatched. Average daily temperature during the field season ranged from 0 - 15° C, although it was common to have fluctuations outside this range. Daylight is almost continuous through the summer months. Average precipitation from May-July is 3.3 cm/month, however in 2011 we had above average amounts of rain, resulting in several nests flooding; we did not categorize these as abandoned nests.

4.3.2. Field Protocol

We located nests by flushing broody males or, rarely, laying females. Phalarope nests consist of simple scrapes in the ground, with a small amount of vegetation used as lining. Eggs in complete clutches were floated to estimate the initiation date of the clutch (Liebezeit et al. 2007). Egg length and width were measured using calipers; from these measurements we calculated egg volume using a phalarope-specific equation volume = 0.44077*length in cm*(width in cm)^2+0.211 (Whitfield 1990). As nests were often found midway through incubation, and eggs lose mass over time, we did not find egg mass to be a useful measurement of egg size. We caught males at the beginning of the season with mist-nets and hoop nets, and during incubation with nest-traps and bow-nets. Males were banded with a metal band and a unique combination of 3 colour bands. Culmen and tarsus length were measured with calipers to the closest 0.1 mm, and wing length was measured with a wing ruler to the closest 0.5 mm, following the protocols outlined in Gratto-Trevor (2004). Male mass was measured using a Pesola scale to the nearest 0.5 g.

Weather data were logged by an automated weather station located on the study plot that took hourly measurements of air temperature, humidity, and wind speed.

Temperature logs have been used to measure incubation scheduling in shorebirds, including little stints (Tulp et al. 2009), red phalaropes, curlew sandpipers (Tulp & Schekkerman 2006), piping plovers (Schneider & McWilliams 2007), pectoral sandpipers (Cresswell et al. 2004) and several species of calidrid sandpipers (Norton 1972); none of these studies report negative effects of instrumentation. Schneider & McWilliams (2007) compared instrumented and uninstrumented nests, and found no difference in hatching success or predation rate. In order to minimize predation and therefore maximize the amount of incubation data gathered, we protected completed clutches using predator exclosures. These consisted of circular wire cages placed over the nest, with a mesh size that enabled adult phalaropes to easily enter and leave, but prevented larger animals such as jaegers or foxes from accessing the nest (Schamel et al. 2004b). No protected nests were depredated, and no signs of adult mortality due to the exclosures were observed.

We used two generations of data loggers: HOBO U23 and HOBO H8 (Onset, Massachusetts, USA), both of which consist of a temperature sensor probe connected to a data storage unit by a plastic enclosed wire. We covered the wire and the storage unit with vegetation to make them less visible. Sensors were inserted through the side of the nest and held in place in the middle of the clutch with a piece of plastic pushed into the ground. Temperature was measured every 30 seconds. The top of the sensor was positioned slightly lower than the top of the eggs, resulting in the tip of the probe being close to, but not directly touching, the brood patch. Absolute incubation temperature measured varied among males, likely due to slight differences in the position of the temperature probe, however male presence and absence could be easily seen as large, rapid changes in nest temperature.

4.3.3. Incubation Load Experiment

To increase the possibility of finding an effect of egg size on incubation behaviour, we artificially increased incubation load using three different methods: (1) by switching a four egg phalarope clutch with a western sandpiper clutch (n=2, average increase in incubation load=3.1cc), (2) by adding a dead sandpiper egg to a three egg phalarope clutch (n=7, average increase in incubation load=6.8cc) or (3) by adding a quail egg to a three egg phalarope clutch (*Coturnix japonica*, n=2, average increase in incubation load=9.9cc). Incubation load was increased for 3-4 days, then clutches were switched back or extra eggs were removed. We switched from using whole egg clutches and quail eggs to adding dead sandpiper eggs due above average abandonment rates.

4.3.4. Analyses

We used Rhythm and Raven Pro 1.4 to semi-automate the process of classifying recesses (Cornell Lab of Ornithology). Rhythm converts files containing date, time and temperature data into sound files, and automatically selects incubation recesses based on selected parameters such as duration and amplitude of changes in nest temperature. We classified a drop in temperature of more than 5 degrees for more than 3 minutes to be a recess (e.g. Camfield and Martin 2009; Smith et al. 2012b). The files produced by Rhythm can be visually inspected in Raven, originally an acoustics program. We proofed the entire dataset in Raven by visually assessing the fit of recess selections made by Rhythm with the temperature recordings. Due to the cold ambient temperature in Nome, it was usually easy to see on the temperature recordings when males left or returned to their nests, however we manually made corrections when necessary. We excluded temperature recordings from the three days prior to hatch, as recesses were less clearly defined due to males changing their incubation behaviour when the eggs began to pip. As we only started recording once a full clutch was reached, we could use all of the data from the beginning of the recording. During nest checks, we occasionally found probes that had been moved from their position in the middle of the clutch, particularly when males built up their nests and buried the probes following flooding in 2011. This also occurred sporadically in both years if males moved the position of their eggs. When found, this was noted, and the temperature records examined. It was generally easy to tell when this occurred, as the maximum temperature on the records dropped substantially. In instances when this was less clear, records were discarded back to the previous nest check when the probe was known to be in the proper position.

Experiment analyses. For the 12 males where incubation load was manipulated, we determined the total percent of time males spent off the nest over six hour periods during experimental and non-experimental periods, using the recesses calculated by Rhythm. Time blocks were classified as experimental when incubation load (total volume of eggs being incubated, in cc) was increased for more than half the duration of the block. Although many shorebirds exhibit diel patterns in incubation behaviour, there was no bias in the time of day measured between experimental and non-experimental periods, therefore this effect should not differ alter our results (e.g. Cartar & Montgomerie 1985; Smith et al. 2012a). To test the effect of changing incubation load within birds, we used a linear mixed effects model (lme in Program R) on the logit transformed percent of time males spent off the nest. We also included wind, a principal components analysis of temperature and relative humidity (due to high correlation between these two variables), male mass, day of incubation and year. Bird ID was included as a random factor to account for multiple measurements per individual. To account for potential temporal non-independence, we included an auto-regression (AR1) auto-correlation structure.

Abandonment analyses. We classified the fates of 62 nests for which incubation behaviour, nest characteristics, and male morphometrics were known. We determined nests to be abandoned when temperature records showed that no incubation had occurred for more than three days, and no male had been seen for two or more nest visits. Several nests flooded in 2011; these nests were not classified as abandoned.

To predict abandonment, we created a candidate model set of 25 models (including a null and a global model) using generalized linear models with a binomial distribution (Table 4-1). The variables we considered were: windtemp (a variable combining temperature and windspeed), relative humidity, incubation load, male mass, male wing length, initiation date, percent of time off the nest, average recess length, and year. Values were averaged over the period when the nest was instrumented. We used an information criterion approach to select the best models, calculating Aikaiki`s information criterion for small sample sizes (AICc) and AICc weights for each model. We calculated AICc weights after more complex versions of the top model that increased ΔAICc by ≤ 2 for each added parameter, and did not increase log likelihood were excluded (Arnold 2010). All analyses, excluding the incubation recording analysis, we done in R 3.0.2 (R Development Core Team, 2013).

4.4. Results

4.4.1. Experimental

The percent of time a male spent off the nest was significantly affected by wind speed, the day of incubation and year, while male mass had a marginally significant effect (Table 4-2). For every increase in wind speed of one metre per second, males increased the time spent off the nest by 2.5% (Fig 4-1). For every two days of incubation, males decreased the percent of time off the nest by one percent (Fig 4-1). Males spent an average of 7.5% more time on the nest in 2012. Heavier males spent more time on the nest, with every two grams of extra mass translating to one percent more time on the nest (Fig 4-1). Our results did not support the hypothesis that incubation load affects incubation behaviour, as the estimated effect size was small and not significant (Fig 4-1).

4.4.2. Abandonment

Abandonment was best predicted by incubation behaviour: the top model was average recess length (AICc weight=0.69) and the second best model was percent of time off the nest (AICc weight=0.18, Table 4-3). No other models were supported (AICc weights <0.06). For every one minute increase in average recess length, the odds of abandonment increase by 7%, and for every one percent increase in percent off, the odds of abandonment increased by 15% (Table 4-3).

4.5. Discussion

Our results suggest that male red-necked phalaropes vary their incubation behaviour according to their energetic requirements and the needs of their developing embryos. We found no evidence that our experimental manipulation of incubation load affected incubation behaviour. However, based on larger samples of unmanipulated nests, males with higher incubation loads take longer recesses (Chapter 2), and we show here that the likelihood of nest abandonment increased with increasing average recess duration and the percent of time spent off the nest.

4.5.1. Experiment

Males left the nest more as windspeed increased, which we interpret to be a result of decreased foraging efficiency due to lower insect activity, increased energetic costs due to convective heat loss to the environment, or both. For every increase in wind speed of 1 m/s, males spent an extra 2.5% of time off the nest (mean wind speed±SD=3.3±1.6m/s, range=0.8-10.4m/s). Thus, based on the extreme values in our dataset, on the windiest days nest attendance could decrease by 24% compared to calm days. Terrestrial arthropod activity has been show to decrease with increasing windspeed, which would result in decreased foraging efficiency (Tulp and Schekkerman 2008). Despite most terrestrial studies of arctic insect activity showing temperature to be the main influence (e.g. Bolduc et al. 2013), we found no effect of the temperature/RH PCA on incubation behaviour. However, little is known about the effect of climatic variables on food availability in the small freshwater ponds where phalaropes forage during the breeding season (Walpole et al. 2008). In addition to affecting food abundance, lower temperatures have been shown to increase energetic requirements during incubation, possibly due to increased egg cooling rate (Reid et al. 2002; Cresswell et al. 2004). However, unlike many shorebirds that nest directly on the ground, we found many red-necked phalarope nests built in areas of dense sedge or thick moss and therefore not directly in contact with the ground. This difference in nest microhabitat could decrease the effect of ground temperature on male incubation and thermoregulatory costs. This is consistent with work by Tulp et al. (2012), who found that smaller shorebirds, including red phalaropes, have more insulated nests. Alternatively, the lack of a temperature effect could be the result of small sample size, as we did find an effect of temperature in the larger non-experimental sample analyzed over the course of incubation (Chapter 2). Effect of temperature could also be partially explained by between-year differences: although 2012 was significantly colder and wetter than 2011, two severe storms in 2011 could have had a greater influence on incubation behaviour (Chapter 2).

The percent of time males spent off the nest decreased with day of incubation, a behaviour seen in diverse avian groups (e.g. passerines, Cooper & Voss, 2013; galliforms, Jia et al., 2010; and charadriforms, Skrade & Dinsmore, 2012). The exact reasons for this pattern are unknown, but may be due to increased embryo sensitivity to fluctuations in temperature with age or, in the case of phalaropes, improved foraging efficiency for incubating males later in the season due to increased prey abundance. Males may also be increasing their investment in the clutch as its reproductive value increases (Delehanty & Oring 1993; Johnston 2011).

Male mass had a negative, marginally significant effect on nest attendance (p=0.08). Heavier males spent more time on the nest, at a rate of approximately 1% higher attendance for every additional 2 grams of male mass. Male mass showed a 12.5 gram range between the lightest and heaviest bird (29-41.5g), which would translate to a difference of 6% in the amount of time off the nest. Greater energetic reserves may allow heavier males to be more selective in the timing of their recesses, and avoid foraging during poor weather when foraging efficiency is low (Piersma et al. 2003).

Despite our previous finding that higher incubation loads in unmanipulated nests increased the total percent of time males spend off the nest and recess length (Chapter 2), we found no effect in birds with experimentally increased variation in incubation load. This may be due to small sample size, as the experimental group was considerably smaller than the non-experimental group (12 vs 50 nests). If incubation load has an effect on male incubation behaviour, and this effect is small, it might be less evident at the time scale used for the experimental analysis (6 hours) compared to the time scale used for the non-experimental analysis (entire incubation period where nest was instrumented). If the effect size of our incubation load manipulation was present but small, our experimental period (ca. 3 days) might be too short for an behavioral change to be measurable. Future experiments to assess the effect of incubation load on incubation behaviour should be done at a larger scale and for longer periods in order to maximize the chances of any effects being measurable.

4.5.2. Abandonment

Abandonment occurred in 15 % of all nests, a rate similar to that found by Reynolds (1985) in red-necked phalaropes, and by Colwell and Oring (1988) in Wilson's phalaropes, but higher than that found by Smith and Wilson (2010) in red phalaropes. While small egg size in multiclutching shorebirds has been considered the result of a trade-off between egg size and egg number, the "small male hypothesis" supported by the work of Lislevand & Thomas (2006) states that in multi-clutching shorebirds where the males incubate and are smaller than females, eggs are sized to match the size of males' brood patches. Our results suggest that egg size not only affects how the eggs fit under the male, but also the energetic requirements of incubation. As shown in Chapter 2, males with higher incubation loads took longer recesses, and we show here that such males were more likely to abandon their nests. In addition, they also initiate their nests earlier in the season. If both incubation load and early nesting increase the chances of abandonment, this could explain why female phalaropes lay smaller eggs in their first clutches compared to replacement or polyandrous clutches later in the season (Schamel et al. 2004a) Males who spend a greater proportion of time off the nest are smaller, however incubation load appears to have a greater effect on the percent of time spent off the nest in larger birds. Thus abandonment is more common in lighter males, those who nest early in the season and, indirectly, those with higher incubation loads. Together, these factors suggest that abandonment results from an energetic deficit due to low reserves (lighter males), lower prey abundance (early season nesters) and males in more demanding situations (higher incubation loads). Incubation load, through its effect on incubation behaviour, appears to be a predictor of abandonment, which could drive the evolution of small egg size in this species.

4.5.3. Conclusions

Our results give mixed support to the “small male” hypothesis. Our experiment showed no effect of incubation load on the percent of time males spend off the nest, however our sample size was small and our treatment was of short duration. Our analysis of the predictors of abandonment does support the hypothesis that males with larger incubation loads are less able to cope with the demands of incubation, which provides support for reduction in egg size being a selective mechanism favouring females laying smaller eggs. Future experimental studies with larger sample size and higher variation in incubation load, over a longer period, and accounting for other factors such as predator abundance would be useful to refine the magnitude to which male incubation behaviour is affected by incubation load, and therefore could provide an explanation for the evolution of small eggs relative female body size in the red-necked phalarope and other polyandrous shorebirds.

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4.7. Figures

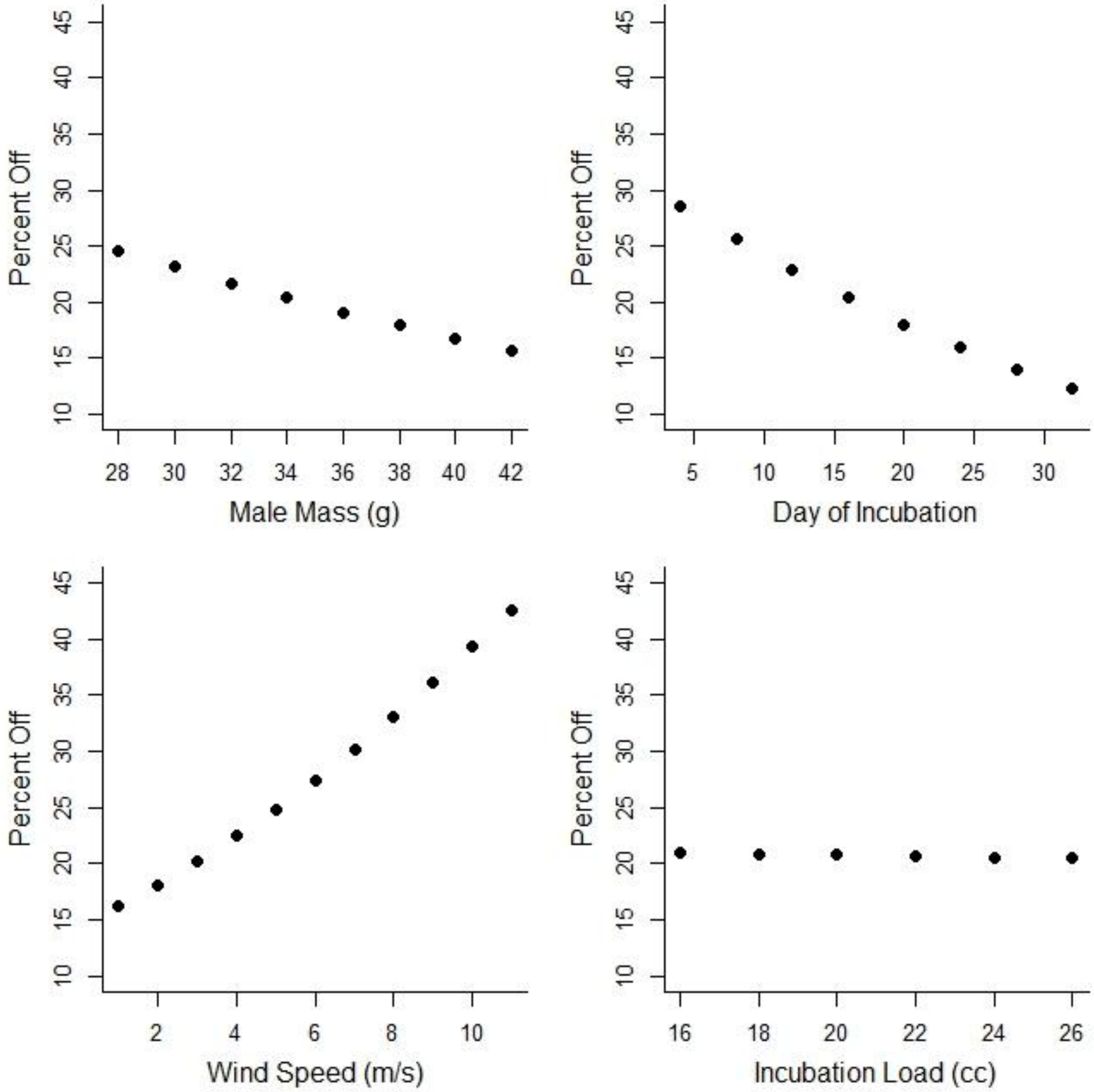

Figure 4-1. Estimates from the lme of effect size of variables that significantly affected the percent of time male red-necked phalaropes spent off the nest during six hour periods, in contrast to the effect of incubation load, which was not significant. Day of incubation is the number of days since the start of incubation.

4.8. Tables

Table 4-1. Candidate model sets for predictors of abandonment. “Abandon” is a binary variable, whether or not the male later abandoned the nest. “Windtemp” combines the effect of wind and temperature, calculated by: average daily low temperature in °C (centrered) – average daily high wind speed m/s (centered). “RH” is the relative humidity. “Inc load” is the total volume of eggs being incubated in cc. “Mass” is male mass in grams. “Wing” is male wing length in millimeters. “Init” is the date of nest initiation, expressed as days since May 1. “Year” is categorical (2011 or 2012). “Percent off” is the proportion of time a male spent off the nest over the entire incubation period monitored. “Xoffmin” is average recess duration, in minutes.

Models
1
windtemp + xRH + toteggvol + mmass + mwing + jinit + percentoff + xoffmin + year
toteggvol + mmass + mwing + jinit + percentoff + xoffmin + year
windtemp + xRH + mmass + mwing + jinit + percentoff + xoffmin + year
windtemp + xRH + toteggvol + jinit + percentoff + xoffmin + year
windtemp + xRH + toteggvol + mmass + mwing + percentoff + xoffmin + year
windtemp + xRH + toteggvol + mmass + mwing + jinit + year
windtemp + xRH
mmass + mwing
percentoff + xoffmin
percentoff + xoffmin + toteggvol
windtemp + xRH + jinit + percentoff + xoffmin + year
windtemp + xRH + mmass + mwing + jinit
toteggvol + mmass + mwing
windtemp + toteggvol + mmass + mwing + percentoff + xoffmin + year
windtemp + toteggvol + mmass + mwing + percentoff
windtemp + toteggvol + mmass + mwing + xoffmin
toteggvol + mmass + mwing
toteggvol + mmass + mwing + percentoff
toteggvol + mmass + mwing + xoffmin
mmass + mwing + percentoff
mmass + mwing + xoffmin
Toteggvol
Percentoff
Xoffmin

Table 4-2. Summary of linear mixed effects model predicting the percent of time males spend off the nest over a six hour period. Bird identity was included as a random effect. Weather is a PCA of temperature and relative humidity, wind is wind speed in m/s, day of inc is the number of days since the start of incubation, year is either 2011 or 2012, male mass is the mass of the incubating male in grams, and incubation load is the total volume of eggs being incubated in cc. N= 12 males, n= 681 6 hour time periods)

Parameter	Estimate	SE	DF	t-value	p-value
Intercept	0.654	0.750	665	0.872	0.383
Weather	-0.015	0.043	665	0.358	0.720
Wind	0.134	.033	665	4.09	<0.0001
Day of inc	-0.037	0.012	665	-3.00	0.003
Year	-0.450	0.145	9	-3.10	0.013
Male mass	-0.040	0.021	9	-1.95	0.083
Incubation load	-0.0004	0.015	665	0.0240	0.981

Table 4-3. Summary of top logistic models predicting nest abandonment in red-necked phalaropes. AICc is Akaiki's information criteria adjusted for small sample sizes. AICc weights were calculated after models adding only uninformative parameters were excluded. The Wald chi-square test was used to calculate X^2. Recess length is the average length of recesses, percent off is the total percentage of time spent off the nest.

Model	AICc	AICc weight	Estimate ± SE	X^2	$P > X^2$	Likelihood ratio
Recess length (min)	48.05894	0.69	0.07269±0.02822	6.6	0.010	1.075
Percent off	50.77876	0.18	0.14303±0.07243	3.9	0.048	1.154

Chapter 5. General Conclusions

Polyandry is rare in avian species, and the selective factors driving its evolution are not well understood. In this thesis, I applied predictions from general reproductive theory to the polyandrous red-necked phalarope in order to better understand how mating systems can influence reproductive traits.

5.1. General Summary

5.1.1. Chapter 1

The Trivers-Willard Hypothesis (1973) states that females should bias their offspring sex ratio depending on their own condition: females in good condition should bias towards the sex with higher variation in fitness and higher intrasexual selection, and females in poor condition should bias towards the sex with lower variation in fitness and lower intrasexual selection. In the polyandrous red-necked phalarope, we predicted that females in better condition (as measured by total volume of egg investment) would produce more female offspring. However, we found the opposite: clutch sex volume increased with sex ratio (males/total offspring). There are several possible explanations for this unexpected result. Our assumption that females have higher variation in fitness because some are polyandrous and some are not may be false. Differential male incubation ability, and sperm storage between clutches by polyandrous females may mean that variation in male fitness is higher than assumed. Even if female variation in fitness is higher, male fitness might be more highly affected by differential investment in eggs, in which case females would benefit from allocating more resources to male-producing eggs. Alternatively, clutch provisioning may not be a good measure of a female’s ability to produce quality offspring, although Schamel et al. (2004) found that egg size was larger in both first and second clutches of polyandrous females compared to females who only mated once.

At a population level, male and female-producing eggs differed in density: female eggs were heavier for a given volume. As we found no difference in shell thickness between male and female-producing eggs, this suggests differences in the ratio of other egg components, namely albumen and yolk. Female eggs, being denser, may contain more albumen, which has a higher density than yolk (Akashi et al. 1997). Our results are consistent with Chin et al. (2012), who found that eggs containing males had a higher ratio of albumen to yolk in the ring-billed gull, a species will male-biased size dimorphism. Male eggs, being less dense, may contain proportionally more yolk, which may increase chick survival, particularly in the first days after hatch (Galbraith 1988). Fisher's Principle (1930) states that if the costs of producing male and female offspring differs, sex ratio should be adjusted to the point where there is equal provisioning to each sex. Our results show a significant relationship between egg volume and clutch sex ratio, and suggest that egg provisioning differs between sexes, however we found no evidence that the population sex ratio differs from 50:50 despite a large sample size. As sex ratio skews have been found in other shorebirds, it is likely that such an adaptation is possible (e.g. Andersson et al. 2003; Reneerkens et al. 2005; Jaatinen et al. 2010), our results suggest that there has not been selection for biases in population sex ratio in this species. Provisioning costs may be less sex-biased than our results suggest, or the costs of biasing sex ratio might outweigh the benefits.

Biases in sex ratio and sex allocation have been convincingly shown in several species, however other studies have failed to find biases where theory suggests they should exist. The former studies show that biases are possible, while latter show that sex allocation theory is not fully developed. Our results highlight the importance of testing assumptions in sex allocation theory: female phalaropes have been assumed to have more variation in fitness due to their polyandrous mating system, sex-role reversal, larger body size, and brighter colouration, however this may not be the case. However, perhaps the largest barrier to the development of sex ratio and allocation theory is our lack of understanding of the mechanism by which primary sex biasing in birds occurs. Without knowing the mechanism or the cost of these biases, it seems unlikely that we will be able to predict when biases are likely to occur.

5.1.2. Chapter 2

Parents must balance the needs of their offspring against their own condition and likelihood of survival. In red-necked phalaropes, where care is male-only, incubation behaviour appears to be mainly governed by the male's energetic balance: small males, who presumably have fewer reserves, spent more time off the nest, as did males exposed to more inclement weather, those with higher incubation loads, and those incubating earlier in the season when food is likely less available. Energetic deficits in males that subsequently abandoned may explain why they showed different incubation behaviour than males who did not abandon. This finding has conservation implications, as changes in the factors that affect incubation could potentially result in males being less able to complete incubation. As changes to climate and food supply during breeding and migration seem likely in the rapidly warming arctic, breeding success may suffer. As one of the smallest uniparental arctic-breeding shorebirds, red-necked phalaropes may be among the most affected by increased variability in factors affecting energetic balance. Together with disturbance from industry, vulnerability to changes in weather and food abundance may lower breeding success and have negative impacts on population numbers (Liebezeit et al. 2009). This may already be happening, as red-necked phalarope population numbers are thought to be decreasing, especially in some areas, although changes in distribution may account for some apparent declines (Morrison et al. 2001; Andres et al. 2012). Understanding the factors that influence incubation, and how this will affect breeding success, is important for predicting how red-necked phalarope population numbers might change under different climate change scenarios.

5.1.3. Chapter 3

Multi-clutching shorebirds lay smaller egg size relative to female body sizes compared to related single-clutching species (Ross 1979; Saether et al. 1986; Liker et al. 2001). The small male hypothesis attributes this to limited male incubation ability (Lislevand & Thomas 2006). Males in multi-clutching shorebirds, especially those with polyandrous mating systems, are often substantially smaller than females and are often responsible for all parental care (Ross 1979; Saether et al. 1986; Liker et al. 2001).

Male uniparental incubators typically spend upward of 30 percent of their time off the nest in order to forage sufficiently to satisfy their energetic needs (Erckmann 1981). If larger eggs require more energy to incubate, we predicted males would increase the amount of time spent foraging, and therefore the amount of time the nest is exposed to the environment. We did not find a measurable effect of experimental increases in incubation load on the percent of time males spent off the nest. As we did find that incubation load affected the percent of time spent off the nest in a larger, non-experimental sample (Chapter 3), we believe that small sample size and short experimental duration may account for our lack of measureable effect.

If males with larger incubation loads are less likely to successfully incubate their clutches, females who produce large eggs would have lower fitness, selecting for smaller eggs. Our best model for predicting whether a male would abandon was recess length, which increased with incubation load (Chapter 3). Therefore males with larger incubation loads are more likely to abandon their nests. This supports the small male hypothesis, as male incubation ability appears to make larger eggs less likely to successfully hatch. Given mixed results in the literature, more work is needed to determine whether small egg size is better explained by the small male hypothesis (Lislevand & Thomas 2006) or by selection to reduce per egg costs in females (Ross 1979; Saether et al. 1986; Liker et al. 2001).

5.2. Conclusion

Theories formed through observations of species with “typical” mating systems and sex roles can be tested and refined through application to species like the red-necked phalarope with uncommon mating systems. Because of their diverse mating systems, shorebirds in general are ideal for studying reproductive behaviour in an evolutionary sense, and this can also provide important information for conservation. In this thesis, some of our predictions were supported while others were not, suggesting that much remains to be learned about the evolution of reproductive traits and mating systems. Future work testing general theories of sexual selection on the diverse array of mating systems found in the shorebirds will expand our understanding of how similar species who share habitat can evolve very different traits and social systems.

5.3. References

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