Parents respond in sex-specific and dynamic ways to nestling parasites

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Nest ectoparasites can impose significant costs to altricial nestlings that are confined to the nest and are dependent on parental care. These costs are often passed on to parents who may compensate for, or magnify, the direct costs of parasites on their nestlings through adjustments in parental care behaviour. If the effects of ectoparasites on nestlings vary across development, parents would be expected to dynamically adjust their behaviour across time with the possibility that males and females may vary in their responses. Currently, we lack a complete understanding of the potential sex differences and variation in parental care behaviour across the nestling period as a function of offspring parasite infection. Our experimental study compared disinfected and parasitized treatment groups to examine how Northern Fowl Mites (*Ornithonyssus sylviarum*) in nests of the North American Barn Swallow (*Hirundo rustica erythrogaster*) affect parental care behaviour. Specifically, we addressed how provisioning rates and nest attendance behaviours (time spent at the nest) changed in response to ectoparasite infection early (day 7) and late (day 13) in the nestling period, and between male and female parents. Early in the nestling period, female-provisioning rates decreased for parasitized nests while male rates remained the same. Males had higher nest attendance in parasitized nests on day 7 whereas females did not alter their attendance of nestlings as a function of the parasite manipulation. Later in the nestling period, parental care behaviours changed dramatically. Males on day 13 visited parasitized nests more than disinfected nests and female rates were the same across treatments. Both males and females showed greater nest attendance for parasitized nests compared to disinfected nests on day 13. These findings suggest that parasites do affect provisioning and nest attendance behaviours: parental care responses differ between males and females, and are dynamic across the nestling developmental period.

Keywords: Barn Swallow, behaviour, ectoparasites, parasite manipulation, parental care,

43 provisioning, nest attendance, nest, sex differences

Parasites exploit host resources and can elicit significant immune and behavioural responses (Lehmann 1993, Zhong et al. 2005, Roberts et al. 2012). Individuals suffering from parasite infections face trade-offs when finite resources are lost to parasites or are used for parasite defence (Owen et al. 2010). This resource loss can have important fitness consequences for hosts, particularly with more virulent parasites, such as haematophagous ectoparasites that feed on offspring, as has been demonstrated for a wide range of avian species (e.g. Bouslama et al. 2002, Brown et al. 1995a, Fitze et al. 2004, Moss and Camin 1970, Norris and Evans 2000, Owen et al. 2010). Birds with altricial nestlings are particularly vulnerable to ectoparasites that live in the nest material because nestlings are confined to the nest and are completely dependent on parental care (Tripet and Richner, 1997). During development, nestlings are particularly susceptible to parasites as they have limited defences with relatively immature and weak immune systems and no ability to preen or physically remove parasites (Owen et al. 2010, Killpack et al. 2013).

The costs of developing in nests with ectoparasites have been documented in altricial nestlings of many different species and include lower mass and body condition (e.g. Moss and Camin 1970, Saino et al. 1998, Szép and Møller 1999), smaller skeletal size (e.g. Christe et al. 1995, Merino and Potti 1995, Richner et al. 1993), changes in the immune and stress response (Arriero et al. 2015, Brinkhof, et al. 1999, De Lope, et al. 1998, Lobato, et al. 2005, Tschirren and Richner 2006), development of less attractive sexual traits (e.g. Fitze and Richner 2002, Tschirren et al. 2003), and reduced long and short term survival (e.g. Brown and Brown 1986, Fitze et al. 2004a, Merino and Potti 1995, Richner et al. 1993). While ectoparasites can impose many important costs for nestlings, a crucial mediator of these costs is determined by how parents respond to the presence of nest parasites.

Changes in parental care in response to ectoparasites varies dramatically across studies and even different studies of the same species (Table 1). Parents may provide more care to parasitized nestlings to compensate for the costs associated with parasites (Tripet and Richner 1997, Hurtrez-Boussès et al. 1998, Bouslama et al. 2002, Bañbura et al. 2004). A different evolutionary strategy may lead parents to favour self-preservation over costly parental care because parasitized nestlings may be of lower quality and have reduced chances of survival and reproduction compared to potential future offspring (e.g. Møller 1994, Darolova et al. 1997, Avilés et al. 2009). Nest ectoparasites can also feed on adults, so limiting time at the nest can also reduce risk of transmission for parents (e.g. Christe et al. 1996a, Møller 1990, Richner and Tripet 1999).

While there is clear evidence in certain species that parental behaviour is being influenced by nest ectoparasites, in other species, parents exhibit no change in their parental care if nests are infected (Table 1). A lack of behavioural response from parents could be because some species of parasites, or levels of infection, may not cause large impacts on nestling condition, growth, and development (e.g. Thomas and Shutler 2001). It could also be that feeding rates of these species are inflexible and thus are unresponsive to parasite infestation, even if nestlings are facing resource trade-offs between development and parasite defence (Morrison and Johnson 2002, Walker and Rotherham 2011).

While behavioural responses vary across species, there is also evidence that individuals within a species exhibit different strategies for dealing with nest ectoparasites. Several investigators have observed variation in parental care responses between males and females to the presence of parasites in the nest (Avilés et al., 2009; Christe et al., 1996a; Hurtrez-Boussès & Renaud, 2000; Frédéric Tripet, Glaser, & Richner, 2002). Sex specific responses to parasites are

90 predicted as males and females often exhibit differences in parental care behaviour without
91 parasites. Theory predicts that the evolutionary interests, and breeding opportunities of males and
92 females differ, which causes them to evolve and maintain different parenting strategies (Houston,
93 Székely, & McNamara, 2013; Sheldon, 2002). These underlying sex differences in parental care
94 behaviour may be magnified or changed by the presence of parasites and should be taken into
95 account when trying to assess how parents respond to nestling parasite infections.

96 While important sex differences in parental care behaviour have been established, we
97 lack information about how such sex-specific differences may vary across the nestling
98 developmental period as a function of ectoparasite infection. Nestlings are changing dramatically
99 over the course of the nestling period in terms of growth rates (McCarty, 2001), development of
100 the acquired immune system (Owen et al. 2010), changes in the physiological stress response
101 (Sims and Holberton, 2000), regulation of body temperature (Morton & Carey, 1971), growth of
102 flight plumage (Pereyra & Morton, 2001), production of fat stores (Riou & Hamer, 2010), and
103 changes in begging behaviour (Leonard & Horn, 2006). Such developmental changes mean that
104 food and care requirements of nestlings also change across development and parents have
105 adapted, often in sex specific ways, to match these needs (García-Navas, Ferrer, & Sanz, 2012;
106 Sonerud et al., 2014; Steen, Sonerud, & Slagsvold, 2012; Wiebe & Slagsvold, 2014). The effects
107 of the parasites themselves are also likely to be inconsistent across development. Parasites can
108 have large impacts early in development (Saino et al., 1998, Norris and Evans, 2000), and these
109 impacts may differ from those experienced by older nestlings (Reed et al., 2012). Susceptibility
110 to parasites may shift across time as older nestlings develop immune systems that can target
111 ectoparasites and shorten feeding time or even reduce parasite fecundity and survival (Killpack,
112 Oguchi, & Karasov, 2013b; Owen, Nelson, & Clayton, 2010c). However, ectoparasites could

also further stress nestlings later in development as they rapidly reproduce, increasing the intensity of infection over time (Proctor & Owens, 2000).

While many different patterns across and within species exist for how parents adjust their behaviour in response to nest ectoparasites (Table 1), there are several important questions remaining. First, we know little about whether there are sex differences in parental care as a function of nestling parasites. Specifically, experiments that both remove and add specific numbers of parasites have not been conducted in order to systematically study the effect of parasites on male and female contributions to parental care. Second, to fully understand how parasites influence parental care we must determine whether behavioural changes within each sex are static or dynamic across development. This will allow us to determine whether measuring parental behaviour at one time point, as many studies do, is enough to infer the overall direction and intensity of parasite induced changes in parental behaviour. To address these questions, we examined the link between the presence of a haematophagous ectoparasite, the Northern Fowl Mite (*Ornithonyssus sylviarum*), and parental care behaviour in the North American Barn Swallow, (*Hirundo rustica erythrogaster*), using an experimental manipulation of parasites and measuring parental care for both males and females during standardized time points both early and late in the nestling development period.

METHODS

Study System

Barn Swallows are small migratory passerines that form social pairs and exhibit biparental care of altricial nestlings. They nest in loose social groups in human-made structures,

most commonly in barns, where they build mud cup nests and raise 3-6 nestlings per brood and have up to 3 broods per breeding season (Turner, 2006). Colony size for these birds can range from solitary to up to 50 breeding pairs. Breeding within the colonies is not highly synchronous and fertile females are present throughout the breeding season (Turner 2006). Extra pair copulations are quite common and up to 40% of nestlings are sired by a male other than their social father (Safran et al., 2005). Distances between active nests depend on the structure of the breeding site and colony size, but minimal distance is about 2.5 m. Barn swallows are obligatory aerial insectivores and make frequent trips to the nest to provision nestlings with insects (Turner, 2006). The primary nest ectoparasite of this species in North America is the blood feeding Northern Fowl Mite, which lives in nest material and feeds intermittently on nestlings. These parasites overwinter in nests and can easily be manipulated by disinfecting nests or adding parasites collected from other nests (Hund et al. *in press*, Møller 1990). The costs of mite infections have been well established in European subspecies (*Hirundo rustica rustica*), where nestlings that were exposed to mites during development had higher mortality, lower body mass, changes in T-cell mediated immune responses, and reduced feather growth (Møller, 1990; Saino et al., 2002).

Study Design

This study was conducted at 11 Barn Swallow breeding sites in Boulder, Jefferson and Weld counties in Colorado between July and August of 2013. All of the field sites used for this study were horse barns and represented medium sized breeding colonies ranging from 15-40 breeding pairs. Parental care observations were part of a reciprocal cross-fostering experiment focused on the heritability and effects of ectoparasites on feather colour development in the North American Barn Swallow. All nests used in this study had brood sizes of 4-5 nestlings upon

hatching. For the reciprocal cross-fostering experiment, nestlings were paired by hatch date and two of the nestlings were exchanged between paired nests, matching mass between the exchanged nestlings as closely as possible and thus maintaining the size rank order of the nestlings in each nest. When finished, each nest had the same number of nestlings that were close the same size, and in the same size rank order as the original brood.

We captured adult Barn Swallows at each field site using mist nets and targeted night captures. Each individual was marked with both a USGS metal numbered leg band and a unique combination of colour bands. We then conducted visual observations to match individual males and females, and thus breeding pairs, to their respective nests using their unique colour combinations.

We monitored nests every three to four days to determine clutch initiation and every day near the end of the incubation period to determine hatch date. The ectoparasite loads of experimental nests were manipulated by first disinfecting all nests and then adding parasites back into a random subset of half of the nests. We disinfected nests by first temporarily removing nestlings and heating the nest to 125° C using a heat gun (Hund et al., *in press*) on day 2 of the nestling period (day 0 = hatch date). Heating nests took < 5 minutes. Nestlings were returned to the nest when the nest temperature returned to < 29 ° C, usually within 8 minutes after heating. Temperatures during heating and cooling were monitored using a digital infrared thermometer. Nests were then either left disinfected, or had 100 live field-collected mites added to them. This number of mites represents a mild to moderate parasite infection in natural Barn Swallow nests (Hund *unpublished data*). This parasite addition technique is similar to other studies, yet our study differs in that we were able to disinfect nests in both treatment groups in the field (Christe et al. 1996a, Christe et al. 1996b) without the use of chemicals (Møller 1994), thus controlling

for and minimizing the effects of nest disinfection on nestlings. Nest parasite levels were monitored for both treatment groups on days 2, 6, 9, and 12 of the nestling period (Parasite nests $n = 23$, Disinfected nests $n = 22$). Specifically, mites were counted on these days by placing a cupped hand into the nest for 30 seconds and then counting the number of mites on the hand, (a common field technique: see Møller 1994), in addition to counting the number of mites on each nestling as well as the container used to hold each nestling. Both of these mite counts (from the nest and from each nestling) were added together as one mite score for each nest, and were used in this study to look at the effect of treatment on mite intensity.

Behavioural Observations

We conducted parental care observations for 45 nests early (day 7) and 36 of these nests late (day 13) in the nestling period. Altricial Barn Swallow nestlings fledge from the nest in 18-20 days, thus observations on days 7 captures the early to middle stage of development (hereafter referred to as ‘early’) and observations on day 13 captures a late stage of development (hereafter referred to as ‘late’). Barn Swallow nestlings exhibit rapid growth during the nestling period and the 6 days between the two observation periods encompass significant changes in nestling body size, behaviour, and development. In the beginning of the nestling period (day 0 – day 5), nestlings are unable to sufficiently thermoregulate on their own (Marsh, 1980), thus females devote a large portion of parental care to brooding (Sanz & Tinbergen, 1999). As we were interested in male and female differences in provisioning behaviour, we choose day 7 of the nestling period for our first set of behavioural observations as at and after this point during nestling development both females and males devote a large proportion of their care to feeding nestlings. Day 13 represent the last time point when we can safely handle nestlings in order to

accurately quantify nest parasites. Past this point, the risk of forcing nestlings to prematurely fledge from the nest, which often causes mortality, becomes too great.

Behavioural observations took place in the mornings between 0600 and 1000 because this is the most active feeding time for Barn Swallows (Maguire & Safran, 2010). After a 15-30 minute habituation period when parents returned to normal behaviour (no alarm calling) we collected data on parental care behaviour for one hour. We observed only one experimental nest at a time during the observational time period. Parents were observed from as far away as possible while still being able to identify individual colour combinations (approximately 9-10 meters from the nest) using binoculars in order to reduce disturbance. Due to the layout of some field sites, we collected observations from behind a blind or after longer habituation periods (similar to Maguire & Safran, 2010). We counted the number of visits by both males and females to their nests during each observation period. We used a total tally of all visits (male and female combined) as an indication of the full provisioning effort (Maguire & Safran, 2010). An examination of combined visits allows us to determine if parasites influence overall parental care in addition to sex-specific responses. As is typically the case for provisioning behaviour studies, we did not have the ability to precisely quantify the identity and quantity of food items parents were bringing to nestlings during each visit; however, we used visits to the nest as a proxy for provisioning effort (e.g. Thomas and Shutler 2001, Nilsson 2003, Spencer 2005, Avilés et al. 2009). When possible, we also kept track of sanitation behaviour, which involved the removal of nestling faecal sacs by parents. Sanitation behaviours were rare compared to feeding behaviour and typically always preceded feeding behaviour when it did occur. On average, parents performed sanitation behaviour 1.2 times per observation period on day 7 and 1.1 times on day 13. Given the low frequency of this behaviour, we choose not to analyse it as part of this

study. Each visit to the nest by parents has some energetic cost, regardless of the activity, and since the most common activity at the nest for parents was feeding, we believe using the number and frequency of visits during a standard observation length is a robust estimate of provisioning behaviour.

We also recorded data on nest attendance by quantifying the total length of time during the observation period that parents spent at the nest. The time at the nest was not correlated with visit number and we used it as a proxy for other forms of parental care behaviour such as brooding, nest sanitation, preening and guarding. Regardless of the behaviour, we considered time spent at the nest to be an investment in parental care. As with visits, we looked at nest attendance for each sex separately, as well as total time at the nest (male and female combined) to quantify the full parental care nestlings were receiving. Some nests ($n = 9$) were only observed on day 7 due to field constraints or nest failure prior to day 13; thus the sample size for these two observation periods varies slightly.

Ethical Note

Behavioural observations and handling of adults and nestlings were done in accordance with guidelines set by the University of Colorado institutional animal care and use committee (IACUC). All procedures in this study were approved by IACUC (protocol number 1303.02). Birds in both experimental groups did not appear to suffer from nest manipulations or from measurements and blood collection.

Statistical Analysis

We used the statistical package R version 3.1.2 (R core Team 2014) to analyse data collected on day 7 ($n = 45$ nests) and day 13 ($n = 36$ nests, a subset of the 45 nests analysed on

day 7). Using a Welch Two Sample t-test, we compared mite intensity and brood size across treatments for both time points. Both visit frequency and time at the nest were compared using a mixed effects linear model for both day 7 and day 13. We applied general mixed effects linear models using the “nlme” package (version 3.1-115). For each model, we included clutch size and observation date as fixed effects and controlling for site as random effects to account for potential non-independence of nests at the same breeding site. The variables ‘clutch size’, ‘observation date’, and ‘site’ (identity of the breeding site where nests were located) were kept in all final models whether they were statistically significant or not, as we wanted our models to reflect the structure of our data (across sites, dates, and brood size). We report the least square means (LSM) for parasitized and disinfected treatment groups for all significant results. When warranted, we applied log transformations to normalize data distributions; this was done for all nest attendance data. We also report raw means (X) and standard errors (SE) for all nest attendance data. For each time point, we created models for the combined provisioning rate and nest attendance time, as this was the total care being experienced by nestlings. Because we were interested in how parasites were influencing parental care behaviour within each sex specifically, we also created models for females and males separately during each time point for visits and nest attendance. As observed in the past (e.g. Maguire and Safran 2010) it is clear that male and female Barn Swallows exhibit significant underlying differences in their provisioning behaviour. Thus, we modelled female and male parental care behaviour separately. Although our data were part of a reciprocal cross-fostering experiment (broods were of mixed parentage), all nests were subjected to the same treatment and the effect of this manipulation did not influence behaviours of interest in this study (parental care). Hubbard (2015) used the same cross-fostering methods used in our study at the same breeding sites in 2012 and found no significant differences in

nestling mass or mortality between experimental and non-experimental nests. Hubbard (2015) also found that nestling growth rates, final mass, and mortality were not significantly different between exchanged nestlings and those who remained in their home nest.

RESULTS

Nests in the parasitized treatment had significantly higher combined mite scores compared to nests in the disinfected treatment on day 7 (Welch Two Sample t-test: $t_{23,28} = 3.38$, $P = 0.0025$, parasitized mean = 19.88 (SD = 27.38, minimum = 5, maximum = 87), disinfected mean = 0.92 (SD = 2.21, minimum = 0, maximum = 10)) and on day 13 (Welch Two Sample t-test: $t_{23,12} = 4.04$, $P = 0.0005$, parasitized mean = 105.96 (SD = 123.35, minimum = 6, maximum = 380), disinfected mean = 4.00 (SD = 7.96, minimum = 0, maximum = 32)), indicating that our experimental parasite manipulations were effective. Brood size was not significantly different between the treatment groups at either time point, day 7 (Welch Two Sample t-test: $t_{47,9} = 0.732$, $P = 0.47$, parasitized mean = 4.41 (SD = 0.50), disinfected mean = 4.31 (SD = 0.55)), day 13 (Welch Two Sample t-test: $t_{44,72} = 0.45$, $P = 0.65$, parasitized mean = 4.17 (SD = 0.76), disinfected mean = 4.08 (SD = 0.63)). For each parent, visit number was not correlated with time spent at the nest on day 7 (Mixed effects linear model: males: $F_{1,37} = 0.361$, $P = 0.72$, females: $F_{1,37} = 0.046$, $P = 0.83$) and on day 13 (Mixed effects linear model: males: $F_{1,32} = 0.84$, $P = 0.31$, females: $F_{1,32} = 1.54$, $P = 0.221$), indicating that these behaviours are independent from each other.

Day 7 Provisioning: Disinfected nests had significantly more combined visits compared to parasitized nests (Mixed effects linear model: $F_{1,37} = 4.52$, $P = 0.04$, parasitized LSM = 21.95 ± 1.83 , disinfected LSM = 26.93 ± 1.89 , fig 1A). This pattern was being driven by females, who visited disinfected nests significantly more than parasitized ones (Mixed effects linear model: $F_{1,37} = 9.46$, $P = 0.0039$, parasitized LSM = 11.38 ± 0.93 , disinfected LSM = 15.38 ± 0.89 , fig 1C).

There was no significant difference in male visits between parasitized and disinfected nests (Mixed effects linear model: $F_{1,37} = 0.087$, $P = 0.77$, fig 1B).

Day 7 Nest Attendance: There was no significant difference in the combined nest attendance time between treatment groups (Mixed effects linear model: $F_{1,37} = 2.77$, $P = 0.12$, fig 2A, parasitized $X + SE = 22.19 + 3.36$ min, disinfected $X + SE = 13.39 + 1.91$ min). Female nest attendance time was not significantly different between treatment groups (Mixed effects linear model: $F_{1,37} = 3.32$, $P = 0.078$, fig 2C, parasitized $X + SE = 15.17 + 1.96$ min, disinfected $X + SE = 9.83 + 1.64$ min), but males spent significantly more time at parasitized nests compared to disinfected nests (Mixed effects linear model: $F_{1,37} = 4.64$, $P = 0.038$, parasitized $LSM = 0.63 \pm 0.09$, disinfected $LSM = 0.37 \pm 0.09$, parasitized $X + SE = 7.03 + 1.78$ min, disinfected $X + SE = 3.55 + 0.85$ min, fig 2B).

Day 13 Provisioning: During the late nestling period, parasitized nests had significantly more combined visits compared to disinfected nests (Mixed effects linear model: $F_{1,32} = 4.39$, $P = 0.044$, parasitized $LSM = 23.62 \pm 2.18$, disinfected $LSM = 17.55 \pm 2.40$, fig 3A). This pattern was being driven by males, who visited parasitized nests significantly more compared to disinfected nests (Mixed effects linear model: $F_{1,32} = 5.54$, $P = 0.025$, parasitized $LSM = 12.33 \pm 1.20$, disinfected $LSM = 8.25 \pm 1.25$, fig 3B). There was no significant difference in female visits between parasitized and disinfected nests (Mixed effects linear model: $F_{1,32} = 1.07$, $P = 0.31$, fig 3C).

Day 13 Nest Attendance: The combined nest attendance time was significantly greater for parasitized nests compared to disinfected nests (Mixed effects linear model: $F_{1,32} = 4.93$, $P = 0.033$, parasitized $LSM = 0.57 \pm 0.074$, disinfected $LSM = 0.33 \pm 0.076$, parasitized $X + SE = 5.95 + 1.21$ min, disinfected $X + SE = 2.29 + 0.55$ min, fig 4A). This pattern was consistent in both

females (Mixed effects linear model: $F_{1, 32} = 4.92$, $P = 0.034$, parasitized LSM = 0.57 ± 0.074 , disinfected LSM = 0.33 ± 0.076 , parasitized X + SE = 4.64 ± 1.09 min, disinfected X + SE = 1.90 ± 0.55 min, fig 4C) and males (Mixed effects linear model: $F_{1, 32} = 19.88$, $P = 0.0001$, parasitized LSM = 0.33 ± 0.031 , disinfected LSM = 0.131 ± 0.031 , parasitized X + SE = 1.32 ± 0.19 min, disinfected X + SE = 0.39 ± 0.06 min, fig 4B).

DISCUSSION

Early in the nestling period, nests infected with ectoparasites were visited at a significantly lower rate compared to disinfected nests. This pattern was largely driven by females who visited parasitized nests at a lower rate compared to disinfected nests whereas males did not differ in their provisioning behaviour as a function of the experimental treatment. Interestingly, this pattern was reversed later in the nestling period when nestlings were much larger and near fledging; parasitized nests were visited at a higher rate compared to disinfected nests. Males visited parasitized nests 1.6 times as much as they visited disinfected nests during this time period, while females did not differ in the number of visits between treatments.

There are several possible explanations for why males and females responded differently to ectoparasites when it comes to provisioning rates. One potential explanation, as shown in a previous study (Avilés et al. 2009), suggests that males and females may be detecting and responding to different cues of parasitic infestations. These cues could include visible detection of the parasites themselves, or marks left on eggs or nestlings from parasites (Avilés et al. 2009). Such visible cues may be dependent on the intensity of the infection, but should be present from the start of breeding if a nest is infected. Alternatively, parents could be detecting cues of parasite infections from the nestlings themselves as changes in body condition, growth rates, or begging behaviour. Prior research has shown that nestlings will significantly increase their rates

of begging when exposed to parasites in order to replace lost resources (Christe et al. 1996a, Cantarero et al. 2013). Such behavioural changes in nestlings may not be present until later in development when nestlings have been exposed to parasites for a longer period of time. If males and females detect and respond to different parasite cues that are present at different time points in development, this could lead to our observation of temporal sex differences in parental care behaviour.

Another potential explanation for the strong sex differences in provisioning between development stages is a difference in investment strategies for each sex based on the cost of, and opportunities for, current and future reproduction (Trivers, 1972). In bi-parental care species, it is thought that investing in parental care yields fewer rewards for males who face reduced fitness through extra-pair mating, than for females, as they could be caring for offspring that are genetically unrelated (Matysioková & Remeš, 2013; Queller, 1997; Sheldon, 2002). Time spent provisioning nestlings can also result in lost mating opportunities for males (Queller 1997). It is possible that early in nestling development males are provisioning at a minimal rate and do not respond to the presence of parasites. For females, there could be greater advantage in investing in future broods with higher quality nestlings (Ackerman & Eadie, 2003; Horvathova, Nakagawa, & Uller, 2012; Sargent & Gross, 1985), hence this might be one explanation for why females with parasites in their nests provisioned nestlings at a lower rate during the early nestling period and did not alter rates during the latter part of nestling development. Towards the end of the nestling period, males may increase their feeding rate to avoid divorce if breeding success is low (Christe et al., 1996a). In this case males may compensate for the costs of parasites when the probability of fledging is greater (Ackerman & Eadie, 2003). If nestlings survive until day 13 while exposed to parasite infestations, this may indicate to the male parent that they are more fit

than nestlings that do not survive. If this is true, males may gain a greater reproductive benefit by investing more heavily in nestlings with parasites at a later stage of development compared to early in development. To a lesser extent, the same principle may be true for females who go from visiting parasitized nestlings less early in development to visiting them the same as disinfected nestlings later in development.

While provisioning rates changed dramatically between the sexes across developmental time, nest attendance behaviour (time spent at the nest per our hourly observation periods) was similar for males and females both early and late in the nestling period. We predicted *a priori* that parental behaviour might be influenced by parasite avoidance, as blood mites have been shown to have negative consequences for both adults and nestlings (Møller 1990, Morrison and Johnson 2002, Gallizzi et al. 2008). If this were the case, we would expect parents to spend less time at the nest in order to avoid transmission. Instead, the combined nest attendance time early in development was not significantly different between treatments, and males actually spent more time at parasitized nests compared to disinfected ones early in the nestling period. Later in development, this pattern was even stronger; combined nest attendance time was significantly greater for parasitized nests and this same pattern was seen in both males and females who both spent nearly double the nest attendance time at parasitized nests compared to disinfected nests. These patterns suggest that parasite avoidance may not be an important factor driving parental care in Barn Swallows. Parents appear to be attending parasitized nests perhaps at the expense of greater exposure to haematophagous mites (Hurtrez-Boussès & Renaud, 2000).

Prolonged nest attendance may also indicate that parents are prioritizing behaviours besides provisioning to care for parasitized nestlings. Longer nest attendance times align with other studies' findings that parents allocate more time to nest sanitation and to brooding when

their nestlings are infected with parasites (Cantarero et al., 2013; Simon et al., 2005; Frederic Tripet & Richner, 1999). Occasionally, female adults will even decrease sleep to engage in sanitary behaviours in response to ectoparasites (Christe et al. 1996b). We could be seeing evidence of such a trade-off between provisioning and other parental care behaviours in our study when males early in development and females later in development both had higher nest attendance but showed no changes in provisioning between treatments.

Given the differences we found in parental behaviour care behaviour between day 7 and day 13, we would encourage future studies to measure how parasites impact behaviour at even more time points during development. In particular, with newly hatched and very young nestlings (day 0- day 6) as this time period may be important, but was not covered in our study. Future work could also focus on behavioural responses to nest parasites across the entire breeding season, including comparisons among different nestling periods but also among different broods (first compared to second or third).

Conclusions

We examined the parental care responses of male and female Barn Swallows at two times during the nestling period (early and late) as a function of an ectoparasite addition and removal experiment. Parental care responses to parasites are both sex-specific and dynamic across the nestling period. Multiple observations across development for males and females were required to capture the nature of these complex behavioural responses to nest parasites. Future research on nestling begging behaviour and specific nest attendance behaviours for both males and females will further illuminate the trade-offs and costs associated with nest parasites and how they are changing over the course of development. As of yet, there is no definitive pattern of how parasites affect parental care behaviour across species. The lack of a detectable pattern across

species has been attributed to varying life history traits across species such as food sources, habitat, and frequency of reproduction, and to variation in the costs associated with different parasite intensities and species (Christe et al., 1996a; Kölliker & Richner, 2001; A. P. Møller, Allander, & Dufva, 1990; Morrison & Johnson, 2002; Walker & Rotherham, 2011). Another important factor to consider is that by observing differences in provisioning rate across the nestling period and for each parental sex, we are able to take into account the dynamic relationship between ectoparasites and behaviour. Studies that only observe provisioning and measure ectoparasite intensity at one time point during this period and do not quantify behaviour of males and females separately may be missing more complex patterns. Our results demonstrate that parental care changes does change over time for both males and females and thus, that behaviour responses to nest ectoparasites are far from static.

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Figure 1. Mean rate of visits on day 7 for disinfected (white bars, n = 22) and parasitized (gray bars, n= 23) treatments. A) Disinfected nests had significantly more combined (male and female together) visits compared to parasitized nests. B) Females visited disinfected nests at a greater rate compared to parasitized nests. C) Males did not differ between the parasite treatments. Bar graphs represent least square means and standard errors from mixed effects linear models with brood size and date as fixed effects and breeding site as a random effect.

Figure 2. Mean nest attendance on day 7 for disinfected (white bars, $n = 22$) and parasitized (gray bars, $n = 23$) treatments. A) There was no significant difference in the combined (males and females together) attendance time between treatments. B). Female attendance time did not significantly differ between the parasite treatments. C) Male attendance time was greater for the parasitized group compared to the disinfected group. Bar graphs represent least square means and standard errors from mixed effects linear models with brood size and date as fixed effects and breeding site as a random effect.

Figure 3. Mean rate of parental visits on day 13 for disinfected (white bars, $n = 18$) and parasitized (gray bars, $n = 18$) treatments. A) Parasitized nests had significantly more combined (male and female together) visits compared to disinfected nests. B). Females did not differ between parasite treatments. C) Males visited parasitized nests at a greater rate compared to disinfected nests. Bar graphs represent least square means and standard errors from mixed effects linear models with brood size and date as fixed effects and site as a random effect.

Figure 4. Mean nest attendance on day 13 for disinfected (white bars, $n = 18$) and parasitized (gray bars, $n = 18$) treatments. A) Parasitized nests had a significantly higher mean combined (male and female together) nest attendance time compared to disinfected nests. B) Female attendance time was greater for the parasitized group compared to the disinfected group. C) Male attendance time was greater for the parasitized group compared to the disinfected group. Bar graphs represent least square means and standard errors from mixed effects linear models with brood size and date as fixed effects and site as a random effect.

Table 1. Previous research findings related to parental care and nest ectoparasites

Avian species	Nest ectoparasite	Parasites experimentally manipulated?	Males and females observed separately?	Time points Observed	Effect of ectoparasites on parental care	Citations
Barn Swallow (<i>Hirundo rustica</i>)	Tropical Fowl Mites (<i>Ornithonyssus bursa</i>)	Disinfected and parasites added	No	16: Daily	Provisioning rates decrease	Møller 1994
Blue Tit (<i>Cyanistes caeruleus</i>)	Hen Flea (<i>Ceratophyllus gallinae</i>)	Disinfected and parasites added	No	1: Day 13	Increase provisioning rate	Tripet and Richner 1997
	Blowfly larvae (Protocalliphora spp.)	Disinfected	Yes	1: Day 13	Females increased sanitation behavior, males do not change	Hutrez-Bousses et al. 1998
	Ticks (<i>Ixodes ricinus</i>), hen fleas (<i>Ceratophylls gallinae</i>), blowfly larvae (<i>Protocalliphora sp.</i>)	Disinfected	No	2: Day 9 & Day 13	Increase provisioning rate	Bouslama et al. 2002
	Hen Flea (<i>Ceratophyllus gallinae</i>)	Disinfected and parasites added	Yes	1: Day 10	Females increase provisioning and decrease sanitation behaviors. Males do not change.	Tripet et al. 2002
	Blowfly larvae (<i>Protocalliphora sp.</i>)	Disinfected	Yes	1: Day 13	Decrease in total provisioning. Males and females not significantly different	Bañbura et al. 2004

Common Swift (<i>Apus apus</i>)	Louse Flies (<i>Crataerina pallida</i>)	Disinfected and parasites added	No	18: every other day	No effect	Walker and Rotherham 2011
Eurasian Kestrel (<i>Falco tinnunculus</i>)	Carnid fly (<i>Carnus hemapterus</i>)	No: Nest boxes (more parasites) and natural nests	No	1: Day 9	No effect	Fargallo et al. 2001
Great Tit (<i>Parus major</i>)	Hen Fleas (<i>Ceratophyllus gallinae</i>)	Disinfected and parasites added	No	1: Day 13	Males increased provisioning, females did not change	Christe et al 1996a
	Hen Fleas (<i>Ceratophyllus gallinae</i>)	Disinfected and parasites added	Only females	1: Day 12	Sanitation behavior increased. No change with provisioning	Christe et al 1996b
	Hen Fleas (<i>Ceratophyllus gallinae</i>)	Disinfected	No	1: Day 9	No effect	Fitze et al. 2004
	Hen Fleas (<i>Ceratophyllus gallinae</i>)	Disinfected and parasites added	No	1: Day 9	Reduced brooding, no change in provisioning	Gallizzi et al. 2008
House Finch (<i>Carpodacus mexicanus</i>)	Mites (<i>Pellonyssus reedi</i>)	No: early (more parasites) and late in season	Yes	1: Day 11	Decrease provisioning for both males and females	Stoehr et al. 2000
House Wren (<i>Troglodytes aedon</i>)	Blowfly Larvae (<i>Protocalliphora sp.</i>)	Disinfected	No	1: Day 12	No effect	Morrison and Johnson 2002
Marsh Tit (<i>Parus palustris</i>)	Hen Fleas (<i>Ceratophyllus gallinae</i>)	Parasites added	No	1: Day 10-12	No Effect	Nilsson 2003
Penduline Tit (<i>Remiz pendulinus</i>)	Mites (<i>Dermanyssus hirundinis</i>)	Disinfected	No	11: Days 7-17	Decrease provisioning	Darolova et al. 1997

Pied Flycatchers (<i>Ficedula hypoleuca</i>)	Mites (<i>Dermanyssus gallinoides</i>), Blowfly Larvae (<i>Protocalliphora azurea</i>), Hen Fleas (<i>Ceratophyllus gallinae</i>)	Disinfected	Yes	2: day 3 and day 9	Females increased nest sanitation behavior, no change in provisioning.	Cantarero et al. 2013
Tree Swallow (<i>Tachycineta bicolor</i>)	Fleas (<i>Siphonaptera</i>), Blowflies (<i>Protocalliphora braueri</i>)	No	No	1: Day 10	No effect	Thomas and Shutler 2001,
Spotless Starling (<i>Sturnus unicolor</i>)	Carnid flies (<i>Carnus hemapterus</i>)	Eggs cleaned to remove spots left from parasites	Yes	1: Day 4	Males decrease provisioning, females did not change	Aviles et al. 2009