

Parasite-mediated growth patterns and nutritional constraints in a cavity-nesting bird

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Summary

1. Trade-offs between growth and immunity of nestling birds can be influenced by parasites, but the magnitude of these effects may depend on availability of critical dietary nutrients. Owing to their importance for both immune system function and growth, dietary carotenoids have the potential to mediate parasite-induced developmental strategies of avian hosts.

2. The effects of ectoparasitic blow flies *Protocalliphora* spp. and dietary carotenoids (lutein and zeaxanthin) on immune function and patterns of growth in nestling mountain bluebirds *Sialia currucoides* were investigated by combining parasite removal and carotenoid supplementation treatments in a 2 × 2 design.

3. Supplemental carotenoids enhanced nestlings' T-cell-mediated immune response following intradermal injection of phytohaemagglutinin.

4. The effect of carotenoid supplementation on rate of mass gain depended on whether broods were exposed to parasites: among parasitized broods, those receiving supplemental carotenoids gained mass more rapidly than nonsupplemented broods, whereas there was no effect of supplemental carotenoids on growth of mass in broods that had parasites removed. This suggests that additional dietary carotenoids allowed nestlings to compensate for the otherwise detrimental effects of parasites on mass gain. For length of the eighth primary feather at fledging, early and late broods differed in their response to parasitism: early broods showed an increase in feather length when parasites were removed, while nestlings in late broods had shorter feathers in the absence of parasites. We suggest that this may reflect within-season variation in parasite-mediated growth strategies of nestlings.

5. Maternal condition was positively associated with mass, condition and rate of feather growth of offspring under all conditions, and also influenced nestling immunocompetence, but only in the absence of parasites.

6. We conclude that dietary carotenoids alleviate some of the detrimental effects of parasites on nestling birds; however, parasites also appear to specifically influence other growth and resource allocation strategies, and possibly constrain maternal or genetic effects on offspring phenotype, irrespective of dietary carotenoid availability.

Key-words: developmental plasticity, host–parasite interaction, maternal effect, nestling growth, resource allocation.

Introduction

Nest-dwelling ectoparasites reduce the reproductive success and condition of breeding birds, and may compromise development of nestlings (Brown, Brown & Rannala 1995; Simon *et al.* 2004). Blow flies *Protocalliphora* spp. (Hough) have been shown to have particularly detrimental effects on nestlings,

despite evidence that parents may attempt to compensate for parasitism by increasing feeding rates (Triplet & Richner 1997). Nestlings may be adversely affected by parasites such as *Protocalliphora* because of the direct loss of nutrients and reduced metabolic capacity resulting from parasite feeding activities (Simon *et al.* 2004). Interestingly, there is some evidence that parents not only increase feeding rates, but also adjust the composition of food provisioned to nestlings in the presence of blow flies (Bañbura *et al.* 2004). This suggests that parents may be attempting to increase the intake of limiting

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nutrients by parasitized nestlings; however, few studies have examined the importance of specific nutrients for the ability of nestling birds to withstand the effects of parasitism.

Exposure to parasites increases investment in immune function by hosts (reviewed in Sheldon & Verhulst 1996; Lindström *et al.* 2004); however, the effectiveness of the immune response is dependent on availability of critical nutrients (e.g. Lochmiller, Vestey & Boren 1993). Antioxidant compounds such as carotenoids, which are acquired by animals from the diet (Goodwin 1984), may be particularly important, both due to their limited availability and the essential role they play in immune system function (e.g. Saino *et al.* 2003). Antioxidants are also required to combat oxidative stress associated with metabolic activity, including reproductive effort (Bertrand *et al.* 2006) and growth (Hofmann & Eichele 1994). These competing demands for carotenoids may partly explain the observation that increased immune response of nestlings is associated with reduced allocation to growth (Soler *et al.* 2003), and may similarly explain the reduction in growth of nestlings exposed to parasites, as this represents an immune challenge (Bize *et al.* 2003). In addition, parasites may influence specific patterns of resource allocation among different tissues (Saino, Calza & Møller 1998; Szép & Møller 1999), and these parasite-induced allocation strategies of nestlings may depend on availability of critical nutrients such as carotenoids. Thus, if nestling birds face a trade-off between investment of antioxidants in immune response and metabolic processes such as growth, their ability to maintain rapid growth rates will be constrained by the availability of carotenoids, combined with the severity of immune challenges (such as parasitism) experienced during development.

Maternally derived carotenoids deposited in egg yolk affect immunocompetence of nestling birds (Saino *et al.* 2003), and may provide protection from oxidative tissue damage during hatching (Surai & Speake 1998). However, enhancement of yolk carotenoids does not appear to afford nestlings greater resistance to parasitism (Berthouly, Helfenstein & Richner 2007). Some of the variation in immunocompetence among avian broods can also be attributed to variation in the amount of dietary carotenoids available throughout the nestling period (Cucco *et al.* 2006). Consequently, when nestlings are exposed to parasites that have significant effects on growth, carotenoids in the diet may be of particular importance in mediating these impacts via enhanced antiparasite immune defences. To our knowledge, only one study has examined the role of dietary carotenoids in mediating the effects of parasites on nestling birds; however, this study reported no effects of the manipulated parasite, hen fleas *Ceratophyllus gallinae*, on their host, nestling great tits *Parus major*, regardless of carotenoid availability (Tschirren, Fitze & Richner 2003). Moreover, great tits exhibit carotenoid-dependent plumage coloration as nestlings, and appear to preferentially invest dietary carotenoids in feathers (Tschirren *et al.* 2003); this creates additional nutritional demands that may obscure trade-offs between investment of carotenoids in growth and immunity (Peters *et al.* 2007; but see Fitze *et al.* 2007).

The objective of this study was to experimentally investigate the effects of ectoparasitic blow flies and dietary carotenoids on patterns of growth in nestling mountain bluebirds *Sialia currucoides* (Bechstein). Blow fly larvae are common parasites of nestlings in this species (Power & Lombardo 1996); although no studies have examined the impacts of blow fly parasitism on mountain bluebirds, a recent experimental study of eastern bluebirds *Sialia sialis* demonstrated that blow flies compromise some measures of nestling health and growth (Hannam 2006), and therefore these parasites have the potential to influence trade-offs between growth and immunity. We predicted that allocation of resources to different tissues by nestling mountain bluebirds would depend on the presence of parasites, and, if the ability of nestlings to simultaneously resist parasites and maintain rapid growth is limited by the availability of carotenoids in the diet, the effects of parasites on growth of nestlings would be less evident in carotenoid supplemented broods than control broods.

Materials and methods

STUDY AREA AND GENERAL FIELD PROCEDURES

This study was conducted in 2006 on a population of mountain bluebirds breeding in nest boxes in central British Columbia, Canada (51° N, 122° W). The study area consists of open arid grassland with patches of large mature Douglas-fir *Pseudotsuga menziesii* (Mirbel). The site contained 72 pairs of nest boxes mounted on fence posts. Paired boxes were spaced approximately 5 m apart, and adjacent pairs were separated by approximately 200 m. Beginning in late April, nests were checked every other day to determine clutch initiation date and clutch size. The average incubation period for mountain bluebirds is 13 days (Power & Lombardo 1996), and completed clutches were therefore checked daily beginning 12 days after clutch completion to determine hatching date (designated nestling day 0). On day 1, nestlings were weighed (nearest 0.01 g) using an electronic scale, and their tarsi were marked with unique colour combinations using a nontoxic pen, so that individuals could be identified throughout the nestling period. From days 3 to 15, nests were visited every other day to determine nestling mass (nearest 0.125 g, using a spring balance), length of tarsus (nearest 0.01 mm, with digital callipers), and length of the longest primary feather (eighth primary, to the nearest 0.5 mm using a ruler). Nestlings were banded with a numbered aluminium band when they were 13 days old. Adults were captured in nest boxes while feeding nestlings, and were banded with a unique combination of three coloured plastic leg bands and one numbered aluminium band. We measured adult mass (nearest 0.25 g) using a spring balance. Length of tarsus and head-bill (nearest 0.01 mm, using digital callipers) were measured as indices of adult structural size. When nestlings had fledged, nests were collected and stored in sealed bags at room temperature for 14 days; the material was then sifted to extract all blow fly pupae and puparia. Previous studies of blow flies have used a similar method to quantify parasite abundance (e.g. Dawson, Hillen & Whitworth 2005a), and the number of pupae detected represents the minimum level of infestation. Although other parasites may have also been present in nests, blow flies commonly have the most detrimental impact on avian hosts, both due to their large biomass relative to other parasites, and the large volume of blood they extract per feeding (e.g. Simon *et al.* 2003). The number of blow flies per nestling was therefore used as a relative measure of parasite infestation in each nest.

PARASITE TREATMENT AND CAROTENOID SUPPLEMENTATION

Heating and supplementation treatments were combined in a 2×2 design, yielding four treatment combinations. Groups of four nests were matched by brood size and hatching date, and each nest in a group was randomly assigned to one of the four treatment combinations. Parasites were removed from experimental nests by heating nest material in a portable microwave appliance on medium power for 5 min (see Richner, Opplinger & Christe 1993). To avoid moisture loss, experimental nests were placed in a sealed plastic bag prior to heating. Heating treatments were repeated every 2 days, from day 1 until day 13. Blow fly larvae live in the nest material, and only move up to feed on nestlings intermittently (Sabrosky, Bennett & Whitworth 1989); consequently, physical removal of nests from boxes can result in the loss of some of these parasites. Control nests were therefore not removed from nest boxes so as to maintain natural levels of parasite infestation; however, control broods were visited at the same frequency as broods in heated nests. Carotenoids were obtained as commercial dietary supplements, consisting of beadlets containing 5% lutein and 0.2% zeaxanthin (Flora Manufacturing and Distributing Ltd, Canada). Supplemented nestlings each received 750 μg of beadlets suspended in 0.05 mL of sunflower oil, while control nestlings received 0.05 mL of sunflower oil only (see Biard, Surai & Møller 2006). Mock (1991) reported that nestling western bluebirds *Sialia mexicana* require 15 g of food per day during peak growth; as the diet of nestling bluebirds consists primarily of lepidopterans and orthopterans, which are estimated to contain 520 mg kg^{-1} of carotenoids (Olson 2006), each nestling consumes approximately 8 mg of carotenoids per day. A dosage of 750 μg was therefore biologically relevant, as it represented approximately 10% of the maximum daily carotenoid intake of nestling bluebirds. Supplements were delivered to nestlings with a 1-mL syringe every 2 days, from day 1 until day 13.

Parent birds may increase the rate at which they feed nestlings either in response to the presence of ectoparasites (Tripet & Richner 1997), or elaboration of carotenoid-based coloration of nestlings (e.g. gape colour; Saino *et al.* 2000), and this may in turn influence nestling performance (Saino, Calza & Møller 1997). To determine whether parents altered their provisioning behaviour in response to either treatment, we used digital recorders to document nest visits by adults. Recorders were connected to small microphones, which were placed in nest boxes to record begging calls of nestlings. The frequency of begging bouts was used as a measure of the frequency of provisioning trips by adult birds (see Dawson, Lawrie & O'Brien 2005b), although this measure did not account for variation in amount of food provisioned per nest visit. Recordings of at least 1 h (mean = 2.3 h, $n = 42$ nests) were obtained between 08.00 and 16.00 h, when nestlings were 11–13 days old. Feeding rates did not vary with time of day at which recordings were obtained ($R = -0.13$, $P = 0.41$, $n = 42$ nests), and this measure was therefore not corrected for recording time.

IMMUNITY ASSESSMENT

T-cell-mediated immune response (CMI) is associated with resistance of avian hosts to ectoparasites (Tschirren *et al.* 2007). CMI of nestlings was assessed with the phytohaemagglutinin (PHA) skin test, a standard method for measuring the ability of birds to respond to a novel mitogen (Smits, Bortolotti & Tella 1999). When nestlings were 8 days old, 30 μL of 2 mg mL^{-1} PHA-P (Sigma) in phosphate-buffered saline was injected intradermally in the right wing web.

Thickness of the wing web was measured using a thickness gauge (nearest 0.01 mm) immediately before and 24–40 h (± 0.05 SE, $n = 241$) after injection. Each measurement was repeated four times, and the mean of these four measurements was used in subsequent calculations. CMI was initially determined as the difference in thickness of the wing web before and 24 h after injection (Smits *et al.* 1999). To control for possible growth-related changes in wing web thickness between measurements (J.E.G. Smits, pers. comm.), the left wing web was also measured before and 24 h after injection of the right wing, and this difference was then subtracted from the initial CMI value. The average change in thickness of the uninjected wing web was minimal (0.04 ± 0.002 mm), but ranged from 0 to 0.27 mm for all nestlings tested in 2006 ($n = 357$).

STATISTICAL ANALYSES

Mountain bluebirds commonly attempt second broods after successfully fledging young from first broods; to avoid including second breeding attempts in the analysis, we therefore only included nests that were initiated prior to known second broods or re-nests (after a failed first attempt). In addition, only nests that fledged at least one offspring were included in the analyses. Seven of 57 nests failed, and the remaining 50 nests were distributed among treatment combinations as follows: 14 heat/control supplement, 13 heat/carotenoid supplement, 11 control heat/control supplement, and 12 control heat/carotenoid supplement. Growth rate constants for individual nestlings were obtained using a linear model for growth of eighth primary, logistic model for growth of mass, and Gompertz model for growth of tarsus, following Dawson *et al.* (2005b), and average growth rates were subsequently calculated for each nest. Brood means were also used in analyses of immunocompetence and morphometric variables. For nestlings, body condition at fledging was calculated as the residuals from a linear regression of body mass on tarsus length, as these measures were highly correlated over the entire study population ($R = 0.49$, $F_{1,76} = 23.86$, $P < 0.001$). Female body condition was calculated with the same method; however, tarsus length was not correlated with female body mass ($R = 0.14$, $P = 0.28$, $n = 62$), and we therefore used head–bill length as a measure of structural size ($R = 0.46$, $F_{1,60} = 16.39$, $P < 0.001$). CMI was not correlated with nestling body mass ($R = -0.05$, $P = 0.77$, $n = 42$) or condition ($R = -0.14$, $P = 0.36$, $n = 42$) and we therefore did not correct this measure for average mass or body condition of nestlings.

Analysis of covariance was used to assess the effects of parasites and dietary carotenoids on parental feeding rates, nestling growth, immunocompetence and characteristics at fledging. The heating and supplementation treatments were included as fixed factors; all analyses also initially included female condition, brood size and hatching date as covariates, as well as all first-order interactions. Nonsignificant terms were removed using a backward stepwise procedure. We were often unable to capture the attending male, and therefore did not include male condition as a covariate. Mean values are presented ± 1 SE, all statistical tests are two-tailed and the significance level was set at $P = 0.05$. Analyses were performed using SPSS (Norusis 2000).

Results

EFFECT OF HEATING TREATMENT ON PROTOCALLIPHORA INFESTATION

Control nests contained 25.8 ± 3.4 blow fly pupae (range: 0–55), while the average infestation of heated nests was 0.5 ± 0.3

Table 1. Effects of parasite and carotenoid supplementation treatments on immunocompetence and growth of nestling mountain bluebirds. Initial ANCOVA models included parasite and carotenoid treatments as fixed effects, as well as female condition, hatching date and brood size as covariates. Nonsignificant terms were removed using a backward stepwise procedure. Results are presented for nonsignificant interaction terms before their removal from the model

Source of variation	SS	d.f.	F	P
CMI				
Heat	0.001	1, 37	0.078	0.782
Carotenoid	0.075	1, 37	4.63	0.038
Heat × carotenoid	0.017	1, 36	1.05	0.312
Female condition	0.003	1, 37	0.17	0.679
Heat × female condition	0.126	1, 37	7.77	0.008
Error	0.599	37		
Tarsus growth				
Heat	0.009	1, 47	4.56	0.038
Carotenoid	0.001	1, 47	0.063	0.80
Heat × carotenoid	0.002	1, 46	1.04	0.31
Error	0.095	47		
Mass growth				
Heat	0.0001	1, 46	0.042	0.838
Carotenoid	0.001	1, 46	0.788	0.379
Heat × carotenoid	0.006	1, 46	5.71	0.021
Error	0.050	46		
Eighth primary feather growth				
Heat	0.0001	1, 46	0.001	0.984
Carotenoid	0.048	1, 46	0.434	0.513
Heat × carotenoid	0.062	1, 45	0.558	0.459
Female condition	0.584	1, 46	5.294	0.026
Error	5.079	46		

pupae (range: 0–9; Mann–Whitney $U = 18.0$, $P < 0.001$, $n = 53$ nests). The experimental manipulation was therefore effective at removing nest-dwelling parasites; in fact, we did not detect any blow fly pupae in the majority (23 of 27) of heated nests. In contrast, 22 of 23 control nests contained at least one pupa.

NESTLING IMMUNOCOMPETENCE

Nestlings in carotenoid supplemented broods exhibited a stronger immune response than control broods (Table 1, Fig. 1). In addition, we detected a significant interaction between the heating treatment and female condition (Table 1); separate analyses of control and heated nests showed that females in better condition produced more immunocompetent offspring, but this relationship was only significant in the absence of parasites (influence of female condition on nestling CMI in heated nests: $F_{1,20} = 5.51$, $P = 0.029$; control nests: $F_{1,16} = 3.72$, $P = 0.072$). There was no additional effect of hatching date or brood size on immune response of nestlings ($P > 0.10$).

EFFECT OF EXPERIMENTAL MANIPULATIONS ON PARENTAL BEHAVIOUR, NESTLING GROWTH AND SIZE AT FLEDGING

Parental feeding behaviour, measured as number of feeding visits per nestling per hour, was not influenced by either the

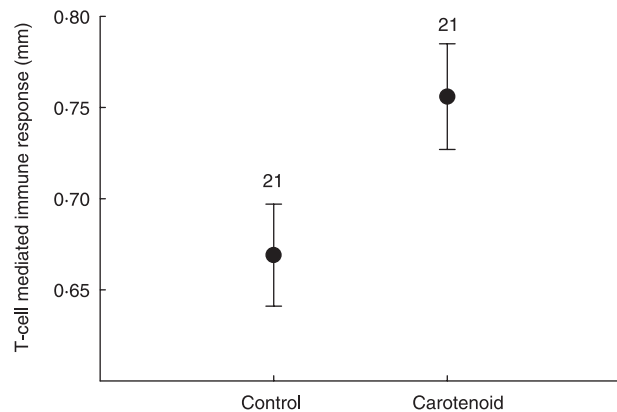


Fig. 1. T-cell-mediated immune response for broods of 8-day-old nestling mountain bluebirds provided with control supplements (control = sunflower oil) or carotenoid supplements (carotenoid = lutein and zeaxanthin in oil) throughout the nestling period. Values are least-square mean \pm SE, calculated after accounting for the influence of maternal condition (see text for details). Sample sizes are shown above error bars.

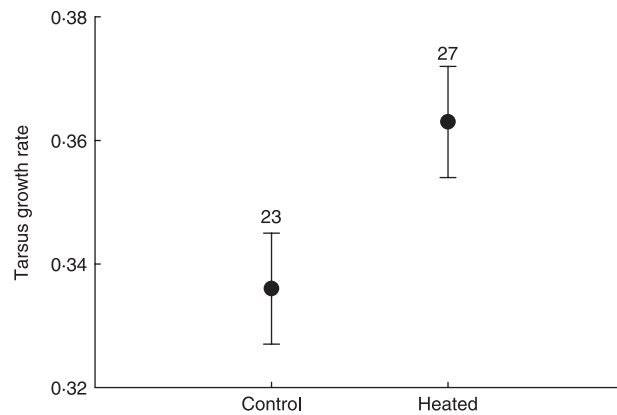


Fig. 2. Mean (\pm SE) growth rate of tarsus for nestling mountain bluebirds in relation to an ectoparasite removal treatment, in which nests were heated to remove parasitic blow flies. Control nests were not manipulated, and were therefore exposed to natural levels of parasite infestation. Sample sizes are shown above error bars.

heating or carotenoid supplementation treatments (heat: $F_{1,38} = 0.015$, $P = 0.90$; carotenoid: $F_{1,38} = 0.86$, $P = 0.36$; heat \times carotenoid: $F_{1,37} = 2.17$, $P = 0.15$), nor did feeding rates vary according to female condition, brood size or timing of breeding ($P > 0.10$). Nestlings grew their tarsi faster in nests that had parasites removed (Table 1, Fig. 2); however, there was no additional effect of carotenoid supplementation on skeletal growth, nor was there an interaction between the treatments (Table 1). The effect of carotenoid supplementation on rate of mass gain depended on whether broods were exposed to parasites (Table 1): among parasitized broods, those receiving supplemental carotenoids gained mass more rapidly than nonsupplemented broods (individual contrast: $F_{1,21} = 4.59$, $P = 0.044$, Fig. 3a), whereas there was no effect of supplemental carotenoids on growth of mass in broods that had parasites

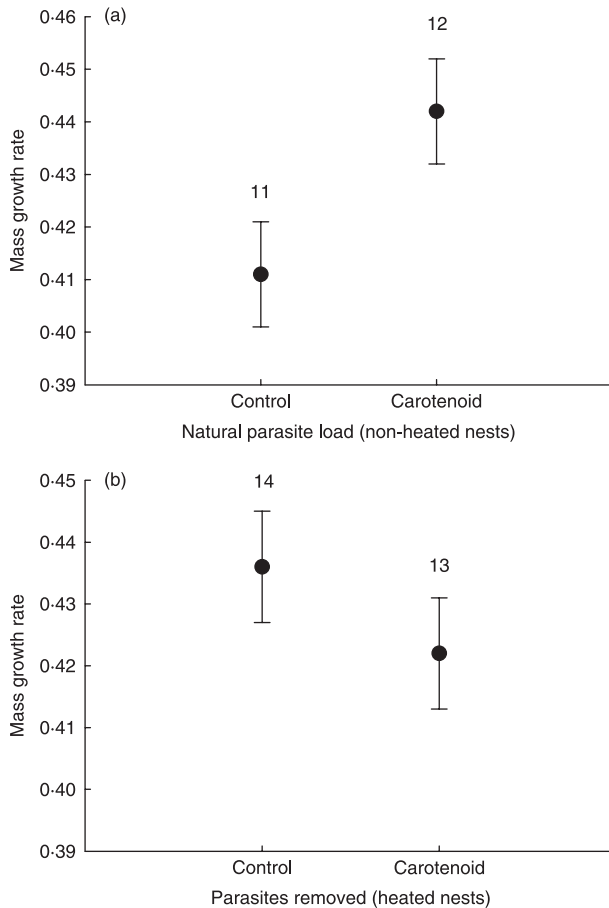


Fig. 3. Rate of mass gain for nestling mountain bluebirds provided with control supplements (control = sunflower oil) or carotenoid supplements (carotenoid = lutein and zeaxanthin in oil) throughout the nestling period. In (a) broods were exposed to natural levels of blow fly parasitism, while in (b) nests were heated to remove blow fly parasites. Mean \pm SE are presented, and sample sizes are shown above error bars.

removed (individual contrast: $F_{1,25} = 1.32$, $P = 0.26$, Fig. 3b). These results suggest that additional dietary carotenoids allowed nestlings to compensate for the otherwise detrimental effects of parasites on mass gain. Females in good condition produced offspring that grew their primary feathers faster, but neither treatment had additional effects on rate of feather growth (Table 1).

We did not detect effects of any variables on the length of tarsus at fledging (Table 2, all covariates $P > 0.41$). Female condition was positively associated with both mass and condition of nestlings prior to fledging (Table 2); however, these traits were not additionally influenced by the parasite or carotenoid treatments (Table 2), or by hatch date or brood size ($P > 0.20$). The parasite removal treatment interacted with hatching date in determining length of eighth primary feathers at fledging (Table 2). To explore this interaction further, nests were classified as 'early' or 'late' based on their position relative to the mean hatching date for all broods in the analysis. Under natural levels of parasite infestation (control nests), chicks hatching early in the breeding season

Table 2. Effects of parasite and carotenoid supplementation treatments on morphological characteristics of nestling mountain bluebirds at day 15. Initial ANCOVA models included parasite and carotenoid treatments as fixed effects, as well as female condition, hatching date and brood size as covariates. Nonsignificant terms were removed using a backward stepwise procedure. Results are presented for nonsignificant interaction terms before their removal from the model

Source of variation	SS	d.f.	<i>F</i>	<i>P</i>
Tarsus length				
Heat	0.045	1, 45	0.228	0.635
Carotenoid	0.001	1, 45	0.003	0.955
Heat \times carotenoid	0.003	1, 44	0.016	0.899
Error	8.844	45		
Mass				
Heat	0.006	1, 44	0.002	0.962
Carotenoid	1.749	1, 44	0.628	0.432
Heat \times carotenoid	0.069	1, 43	0.024	0.877
Female condition	13.315	1, 44	4.783	0.034
Error	122.501	44		
Body condition				
Heat	0.027	1, 44	0.032	0.858
Carotenoid	0.566	1, 46	0.693	0.410
Heat \times carotenoid	0.007	1, 43	0.008	0.929
Female condition	4.337	1, 44	5.307	0.026
Error	35.961	44		
Eighth primary feather length				
Heat	40.227	1, 43	4.695	0.036
Carotenoid	3.173	1, 43	0.370	0.546
Heat \times carotenoid	5.486	1, 42	0.635	0.430
Hatch date	15.420	1, 43	1.800	0.187
Heat \times hatch date	38.477	1, 43	4.491	0.040
Error	368.436	43		

had significantly shorter flight feathers prior to fledging when compared with chicks hatching from late nests (individual contrast, control nests: $F_{1,16} = 11.12$, $P = 0.004$). When parasites were reduced, however, there was no difference in feather length between early and late broods (individual contrast, heated nests: $F_{1,26} = 0.83$, $P = 0.37$; Fig. 4). Moreover, there was a significant effect of parasite removal in both early and late nests, but this effect was in opposite directions: among early nests, parasite removal resulted in increased feather length at fledging, whereas among late nests, feather length was reduced in response to parasite removal (Fig. 4). Importantly, there was no difference in the intensity of parasite infestation between early and late control nests (number of blow flies per nestling in early broods: 5.2 ± 0.8 , late broods: 4.0 ± 0.8 ; independent samples $t = 0.91$, d.f. = 18, $P = 0.38$).

Discussion

Carotenoid supplementation enhanced CMI response of nestling mountain bluebirds, suggesting that in this species, dietary carotenoids are important components of immune function, and their availability in the diet of nestlings is limited. Moreover, parasitized nestlings showed an enhanced rate of mass gain when provided with supplemental carotenoids (Fig. 3a); this indicates that dietary carotenoids have the potential to alleviate detrimental effects of parasites on

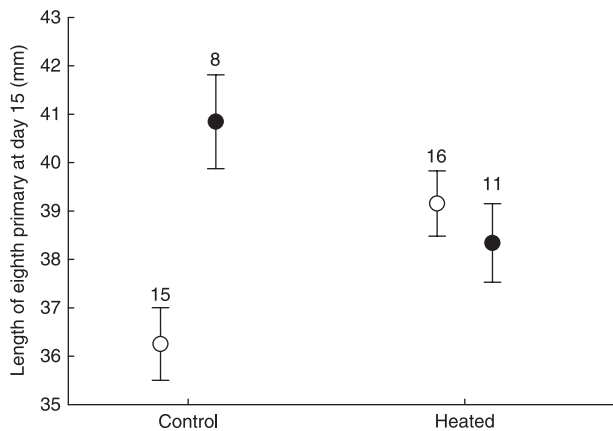


Fig. 4. Mean (\pm SE) length of eighth primary feathers of nestling mountain bluebirds at day 15 in relation to an ectoparasite removal treatment, in which nests were heated to remove parasitic blow flies. Control nests were not manipulated, and were therefore exposed to natural levels of parasite infestation. For each experimental group, broods are separated into early nests (open circles) and late nests (filled circles), relative to the mean hatching date for all broods in the analysis. Sample sizes are shown above error bars.

nestling birds. Similar effects of dietary carotenoids have been reported in captive adult male greenfinches *Carduelis chloris* exposed to experimental immune challenges: induced immune responses were associated with lower rates of mass gain, but this effect was not evident in birds that received supplemental carotenoids (Hörak *et al.* 2006). To our knowledge, however, the present study is one of the first to demonstrate the capacity of dietary carotenoids to reduce the effects of natural stressors such as parasites on development of nestlings in a free-living population of birds.

Although the effect of parasites on growth of mass was alleviated by supplemental dietary carotenoids, similar nutritional constraints were not evident in the analysis of feather length at fledging. Instead, nestlings produced early in the breeding season grew shorter wing feathers in the presence of parasites, regardless of carotenoid availability, whereas late-hatching broods exhibited increased feather investment in response to parasitism (Fig. 4). Notably, these patterns could not be explained by seasonal differences in intensity of parasite infestations in control nests. Adaptive allocation to wing growth in the presence of parasites has been reported in several bird species (e.g. Saino *et al.* 1998; Szép & Møller 1999), and may facilitate early fledging, though at the potential cost of feather quality (Dawson *et al.* 2000). For late-hatching broods, our results provide evidence for a similar parasite-mediated allocation strategy in mountain bluebirds (Fig. 4). In contrast, for early broods, reduced allocation to flight feathers in the presence of parasites may be interpreted as a consequence of energetic constraints that are disproportionately experienced early in the breeding season (e.g. temperature stress; Dawson *et al.* 2005b). However, it is also possible that this pattern reflects an alternative parasite-mediated allocation strategy characteristic of early broods, which allows nestlings to resist parasites (e.g. by investing in immune defence), while avoiding future costs of accelerated growth (Metcalfe & Monaghan

2001). Indeed, early hatching parasitized nestlings may be less constrained from prolonging feather development beyond fledging, and may thereby achieve similar wing lengths at maturity relative to parasitized nestlings from late broods (see Bize *et al.* 2003). Results of this study do not allow us to distinguish between these hypotheses, however, and further research is needed to determine the relative influence of energetic constraints and resource allocation strategies on parasite-mediated phenotypes of nestling bluebirds, particularly in the context of intraseasonal variation.

Structural size measures such as tarsus length are highly heritable in birds (e.g. Gebhardt-Henrich & van Noordwijk 1991), and it is therefore not surprising that we did not identify any environmental variables that predicted this trait in nestling mountain bluebirds; however, our results indicate that blow fly parasites reduce the rate at which this final size is attained (Fig. 2). Similar effects of parasites on skeletal growth have been shown in other species (e.g. Szép & Møller 2000). This may represent an important cost of parasitism, as adult structural size is achieved during the nestling period, and more rapid skeletal growth may increase availability of resources for investment in other functions (e.g. feather growth) later in nestling development. It is also not surprising that neither rate of growth nor length of tarsus in this study was influenced by availability of dietary carotenoids, as avian skeletal growth is limited by the availability of other nutrients, namely calcium (Dawson & Bidwell 2005). Interestingly, however, a recent experimental study investigating the effects of blow flies on nestling performance in eastern bluebirds breeding in Pennsylvania did not report similar effects on tarsus growth in this species, despite considerably higher parasite loads in control nests relative to our study population (Hannam 2006). This suggests that mountain bluebirds in central British Columbia may be more limited by dietary calcium availability than bluebirds in eastern North America; as this is unlikely to result from differences in soil calcium availability between these regions (Arocena & Sanborn 1999; Lyon & Sharpe 1999), it may reflect regional (and possibly species) differences in diet composition that merit further investigation.

Pre- or postnatal maternal allocation of resources can affect phenotypic traits, as well as immunocompetence of offspring (reviewed in Bernardo 1996; Saino *et al.* 2003). In this study, we did not detect a main effect of female condition on nestling CMI, but there was a significant interaction between maternal condition and the parasite treatment in this analysis (see Results). This interaction was explained by the fact that offspring of females in good condition exhibited a stronger immune response, but only in the absence of parasites (heated nests), whereas there was no relationship between female condition and nestling CMI in parasitized nests. It is significant that offspring of high-quality females also grew their feathers faster, and were both heavier and in better condition at fledging, regardless of parasite load or carotenoid availability. This suggests that nestlings that were able to allocate more resources to somatic growth, possibly due to pre- or post-hatching maternal effects, or genetic effects, experienced a trade-off between growth and immune function in the presence of

parasites. Prioritization of somatic growth over immunity by parasitized nestlings may represent an optimal strategy in light of such trade-offs (e.g. Soler *et al.* 2003), as this may reduce the amount of time nestlings are exposed to parasites (Saino *et al.* 1998). In particular, allocation of resources to maintenance of body mass and condition despite stressors such as parasites may be important, as these traits commonly predict recruitment and future reproductive success in birds (e.g. Merilä & Wiggins 1995).

In this study, we provide evidence that supplementation with the dietary carotenoids lutein and zeaxanthin enhanced the ability of nestling bluebirds to mount an immune response to a mitogen challenge (Fig. 1). Notably, however, studies of both great tits and blue tits *Cyanistes caeruleus*, which are unusual among birds in exhibiting carotenoid-based plumage coloration as nestlings, have failed to detect effects of these carotenoids on immunocompetence (Biard *et al.* 2006; Fitze *et al.* 2007). This discrepancy highlights the importance of recognizing the range of physiological pathways through which dietary carotenoids may be diverted in different species (e.g. McGraw 2005). However, as the majority of birds do not produce carotenoid-based plumage signals during the nestling period (Brush 1990), the extent to which effects of supplemental carotenoids on nestling tits can be extrapolated to other species is uncertain. In fact, our results indicate that dietary carotenoids such as lutein may indeed be invested in immune functions, providing they are not selectively deposited in developing feathers of nestling birds.

Ecological stressors such as parasites exert effects on their hosts within the context of variation in host immune defences and other energetic costs such as growth, which are in turn influenced by dietary nutrients (Norris & Evans 2000). Results of this study demonstrate that at least some of the detrimental impacts of ectoparasites such as blow flies can be alleviated when sufficient dietary carotenoids are available to their avian hosts, as nestling bluebirds were able to cope with parasites without compromising mass gain when they were provided with supplemental carotenoids. Furthermore, our results demonstrate parasite-mediated growth patterns that, while not influenced by dietary carotenoids, suggest within-season variation in prioritization of feather growth by nestling bluebirds in response to blow fly parasites. Finally, removal of nest-dwelling parasites revealed parasite-mediated trade-offs between immune response and growth of nestlings produced by high-quality females, which suggests that parasites may constrain maternal effects on offspring phenotype. In nestling mountain bluebirds, this constraint was expressed in the maintenance of feather growth and body condition at the expense of immunity, regardless of dietary carotenoid availability. We suggest that, while some growth processes may be maintained in parasitized nestlings with sufficient carotenoids in the diet, future studies should examine the role of other limiting nutrients in alleviating effects of parasites on the growth of nestling birds, particularly as seasonal and geographical variation in diet composition (e.g. calcium availability) may influence variation in responses of avian hosts to the costs of parasitism.

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