Breeding success and lutein availability in great tit (Parus major)

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1. Introduction

Caterpillars are a seasonal food resource for birds, and their abundance varies considerably among years, within season and in different habitats. Caterpillar availability affects the breeding performance of birds through nestling growth, development and survival (Baibura et al., 1994; Tremblay et al., 2003; Isaksson and Andersson, 2007). As prey items, caterpillars require shorter handling times, are easier to ingest and contain more water than spiders for example, and are thus preferred food for growing nestlings (Baibura et al., 1999; Tremblay et al., 2005). Caterpillar abundance decreases in heavily polluted environments, negatively affecting nestling growth and survival in great tit (Parus major) (Slagsvold and Lifjeld, 1985; Eeva et al., 1997b). Not only diet quantity but also diet quality affects the fitness of nestlings and their fledging success. In polluted areas great tit parents have been shown to catch more alternative food, such as beetles (Eeva et al., 2005), because caterpillar numbers are expected to be reduced in the vicinity of point source polluters (see e.g. Slagsvold and Lifjeld, 1985; Eeva et al., 1997b, 2005).

Herbivorous caterpillars are rich in carotenoids (e.g. Partali et al., 1987). Great tits prefer carotenoid-rich caterpillars as optimal food in their diet when feeding their nestlings (Perrins and McCleery, 1989; Eeva et al., 2005). Different insect species have different carotenoid profiles and are available to great tits at different times during the breeding season. For example, autumnal moth larvae (Epirrita autumnata) are rich in lutein while sawfly larvae (Hymenoptera: Symphyta) are rich in β-carotene (Sillanpää et al., 2008). Such temporal variation in the availability of carotenoid-rich diet may affect the fitness of offspring affecting their antioxidant levels, body condition, immune response, survival and phenotype of offspring (Biard et al., 2006). Carotenoids may function as antioxidants against exogenous (e.g. plant allelochemicals) and endogenous stress in caterpillars and they have an important role in bird pigmentation, though their role as antioxidants in birds is not clear (Ahmad, 1992; Isaksson et al., 2005). Yet, what is clear is that carotenoids are often related to increased immune response, regardless of their actual function (Møller et al., 2000; Hidalgo-Garcia, 2006).

Great tit parents are under selection to time their reproduction so that the peak food demand of nestlings coincides with peak caterpillar abundance (van Noordwijk et al., 1995). When caterpillar abundance peaks, the carotenoid availability for growing nestlings should be at the highest level and this could be expected to show up in the carotenoid profile or concentration of nestling plasma. We studied the breeding success and plasma lutein concentration of great tit nestlings in relation to temporal variation in availability of carotenoid-rich prey in the field. Previously, the effects of larval

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availability and their carotenoid concentration to nestling success have been studied e.g. in Partali et al. (1987) and Isaksson and Andersson (2007). The present study is the first one to investigate seasonal change in carotenoid availability in a two-trophic system of insects and great tit nestlings during a longer time span than e.g. studies of Partali et al. (1987) and Isaksson and Andersson (2007). In our study the caterpillar collection was expanded to cover the entire breeding season of the great tit. Simultaneously we measured temporal trends in caterpillar abundance and their lutein concentration. In our study populations carotenoid-rich caterpillars, such as autumnal moths and sawflies, has proven to be important food source for great tit nestlings (Eeva et al., 2005). Our study sites are set along the pollution gradient of a copper smelter, which is known to affect caterpillar abundance: caterpillars are less numerous in the polluted area than in the unpolluted area (Eeva et al., 1997b). We expect that spatial and temporal variation in caterpillar abundance would be reflected in the lutein concentration of nestlings’ plasma. We also expect to find a positive association between larval biomass and fledging success.

2. Materials and methods

The study was carried out in 2004 in twelve established nest box areas at different distances (from 0.5 km to 12 km) from the point source of pollution (a copper smelter) in the town Harjavalta (61°20′N, 22°10′E) (see e.g. Eeva, 1996). The main pollutants in the area are sulphur oxides and heavy metals due to industrial emissions (Kiiikkiä, 2003). Vegetation, insects and birds suffer from the heavy metal contamination (Eeva and Lehikoinen, 2000; Kiiikkiä, 2003). Heavy metal concentrations diminish exponentially from the pollution source and approach background levels at 5 km from the smelter (Eeva et al., 1997a): nest box sites were accordingly divided into polluted sites (6 sites less than 2 km from the smelter) and unpolluted sites (6 sites over 5 km from the smelter). Dividing study areas to the polluted and to the unpolluted sites have two major advances. First by doing so the indirect effects (i.e. secondary effects of pollution) are taken better into consideration than using just concentrations of different pollutants. Secondly the pollution level is not a self evident measure when there are different pollutants, e.g. different heavy metals, with different combinations present in the environment. If we consider heavy metals, some of them may have stronger effect than others. Nest boxes were visited regularly from the beginning of the breeding season onwards. The study was performed under licenses from the Animal care & use Committee of Turku University and Regional Environment Centre.

We focused on lutein among the different carotenoids because it is the predominant carotenoid in plasma and feathers of many bird species, including the great tit (Partali et al., 1987; Isaksson and Andersson (2007)). The present study was performed under licenses from the Animal care & use Committee of Turku University and Regional Environment Centre.

2.1. Extraction and analyses of carotenoids

The larvae were weighed to obtain fresh body mass, then freeze-dried and weighed to obtain dry mass and ground into fine powder. From collected caterpillars, N = 67 autumnal moth and N = 48 sawfly larvae had sufficient dry mass for HPLC analyses. A known amount of powder or a known volume of great tit plasma (10–35 μl, N = 59) was extracted three times with 100% acetone. The solvent was evaporated from the combined extract under vacuum and the residue dissolved into a small volume of 80% aqueous acetone (plasma) or 100% acetone (larvae). The carotenoid composition of the extracts was analysed with high-performance liquid chromatography at 450 nm using either a Merck Purospher STAR RP-18 (55 ± 2 mm, i.d., 3 μm; for plasma) or an YMC C-30 (250 × 4 mm, i.d., 5 μm; for larvae) HPLC column. Standards were used in all carotenoid analyses. Since the samples contain no carotenoid esters, the saponification step in extraction was unnecessary (see Sillanpää et al., 2008 for methodological details).

2.2. Statistical analyses

Statistical analyses were performed with general linear mixed models (GLMM; Mixed procedure in SAS). Degrees of freedom were calculated, as recommended, with Satterthwaite’s procedure (Littel et al., 1996). Variation in the concentration of lutein in caterpillars was analysed with GLMM with area (polluted vs. unpolluted), date, dry mass of larvae and the interactions dry mass × area and date × area as fixed effects and territory as a random factor. The lutein concentration was log10-transformed to meet the assumptions of the models. Variation in total caterpillar biomass was analysed with area, sampling period and the interaction between area and sampling period as fixed effects and territory as a random factor. Variation in proportional (%) body water content of larvae was analysed with GLMM with area and sampling period as explanatory variables, body mass as a covariate and tree as a random factor with territory as a subject variable. The interaction between body mass and study area and interaction between sampling period and study area were included in an initial model, but they were dropped from the final model as insignificant variables (both interactions P > 0.05). Variation in body mass of larvae was analysed with area and sampling period as explanatory variables and tree as a random factor with territory as a subject variable. The effect of total biomass of larvae on nestlings’ lutein concentration was analysed with the mixed model procedure: lutein concentration of nestling plasma was the response variable, with total biomass of all collected larvae as explanatory variable and territory as a subject variable in a repeated statement. The lutein concentration of nestling plasma was log10-transformed to meet the assumptions of the model. The seasonal trend in lutein concentration of nestlings’ plasma was analysed with generalised linear models (GLM: Proc Genmod procedure in SAS with log link function and Poisson distribution). Because there was no significant difference in the lutein concentration of nestling plasma between the polluted and the unpolluted area, study area was eliminated from this model (see Sillanpää et al., 2008). The effect of hatching
date, study area and biomass of larvae on number of fledglings was analysed with generalised linear models (GLM; with log link function and Poisson distribution). Because hatching date had no significant effect on number of fledglings it was dropped from the final model ($\chi^2 = 1.01$, $P = 0.32$).

3. Results

3.1. Carotenoid concentration and biomass of autumnal moths

Lutein concentration of autumnal moth larvae was significantly lower in the polluted than in the unpolluted area ($F_{1,56.8} = 4.40$, $P = 0.040$; Table 1), though the difference is so small (2.5%) that it may not have any biological meaning. There was a declining seasonal trend in lutein concentration of autumnal moth larvae ($F_{1,60.9} = 11.6$, $P = 0.0012$; Fig. 1a). The interaction between the date and study area was significant ($F_{1,59.3} = 7.08$, $P = 0.01$). There was a negative relationship between dry mass of larvae and their lutein concentration ($F_{1,59.3} = 21.1$, $P < 0.0001$), the relative lutein concentration of the smallest (in dry mass) caterpillars being 25.3% higher than that of the largest ones.

The biomass of autumnal moths peaked earlier in the unpolluted than in the polluted area: in the polluted area there were less autumnal moth larvae during the first sampling period than during the second and the third sampling period (a significant interaction between sampling period and area $F_{3,1021} = 4.98$, $P = 0.0019$, Fig. 2a). During the fourth sampling period there was no autumnal

### Table 1

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<th>Autumnal moths</th>
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<td><strong>Polluted</strong></td>
<td>90.12 (79.51–102.14)</td>
<td>18.15 (14.58–22.58)</td>
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<tr>
<td><strong>Unpolluted</strong></td>
<td>90.76 (81.81–100.67)</td>
<td>15.56 (12.01–20.17)</td>
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Fig. 1. a) Lutein concentration (µg/g) of autumnal moth and b) sawfly larvae during the breeding season of great tit. Regression line is plotted in both figures.

Fig. 2. a) Mean biomass (mg/tree) of autumnal moth and b) sawfly larva and c) all larvae and 95% confidence limits during 4 different sampling periods. Black bars indicate the polluted area and grey bars the unpolluted area. The values in figures are back transformed from logarithmic scale. Sampling periods 1: 17th of May–21st of May, 2: 24th of May–27th of May, 3: 31st of May–4th of June and 4: 14th June–17th.
moth larvae present in sampled trees. In the unpolluted area the autumnal moth biomass was highest during the first and the second sampling period and then decreased towards the third sampling period and was zero during the fourth sampling period. The overall autumnal moth biomass was higher during the first and second sampling period than during the third sampling period ($F_{3, 1003} = 9.56, P < 0.0001$, Fig. 2a).

The body mass of autumnal moth larvae increased through the sampling periods ($F_{1, 576} = 7.42, P = 0.0014$), but did not differ between the two areas ($F_{1, 503} = 0.08, P = 0.78$). There was no significant interaction effect on the body mass among study area and sampling period ($F_{1, 576} = 0.03, P = 0.97$).

The mean water content of autumnal moth larvae was 86.6% (SD = 3.59). Small (in body mass) autumnal moth larvae contained proportionally more water than large ones ($F_{1, 59} = 46.27, P < 0.0001$). The water content of autumnal moth larvae did not differ between the polluted and the unpolluted area ($F_{1, 479} = 0.05, P = 0.82$), but it differed among sampling periods ($F_{2, 59} = 3.51, P = 0.036$, Table 2). Overall, larval water content peaked during the third sampling period, when the body mass was controlled for. The first sampling period did not differ from the second sampling period, but it differed from the third sampling period.

### 3.2. Carotenoid concentration and biomass of sawflies

Lutein concentration of sawfly larvae was 14% higher in the polluted than in the unpolluted area ($F_{1, 44} = 4.32, P = 0.044$; Table 1). There was a declining seasonal trend in lutein concentration of sawfly larvae ($F_{1, 46} = 9.64, P = 0.0033$; Fig. 1b). Lutein concentration of the smallest sawfly larvae was 49.2% higher than that of the largest ones ($F_{1, 44} = 9.56, P = 0.0035$). No interaction effect on lutein concentrations was found between study area and dry mass of larvae ($F_{1, 44} = 3.69, P = 0.061$).

The mean biomass of sawfly larvae did not differ between the polluted and the unpolluted area ($F_{1, 1112} = 0.5, P = 0.83$, Fig. 2b). There was a seasonal trend in biomass of sawfly larvae: biomass increased in both areas during the sampling periods being lowest in the first sampling period and highest during the fourth sampling period (Fig. 2b).

The body mass of sawfly larvae did not differ between the study areas ($F_{1, 59} = 1.17, P = 0.28$) but it differed among the sampling periods ($F_{2, 603} = 7.32, P = 0.0003$). Due to growth of the larvae larval body mass was higher during the fourth sampling period than during the second and the third sampling period. There was no interaction between study area and sampling period ($F_{2, 603} = 0.92, P = 0.44$).

The mean water content of sawfly larvae was 85.3% (SD = 4.11). There was a negative relationship between sawfly larval body mass and water content (main effect $F_{1, 42} = 16.2, P = 0.0002$). The water content of sawfly larvae did not differ between study areas ($F_{1, 42} = 0.02, P = 0.89$). There was no temporal change in relative water content during the sampling periods when the body mass was controlled for ($F_{3, 38} = 1.43, P = 0.25$, Table 2).

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<th>Water content (%) of autumnal moth (N = 64) and sawfly (N = 48) larvae and 95% confidence limits (in parentheses). Sampling periods 1: 17th May–21st May, 2: 24th May–27th May, 3: 31st May–4th June and 4: 14th June–17th June. The values are back transformed from logarithmic scale.</th>
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There was a seasonal trend in the total biomass of caterpillars (including all collected larvae). The total biomass increased from the first to the third sampling period and decreased again during the fourth sampling period ($F_{1, 993} = 21.21, P = 0.0001$, Fig. 2c). The total biomass of caterpillars was 51% lower in the polluted than in the unpolluted area ($F_{1, 375} = 33.02, P < 0.0001$, Fig. 2c).

### 3.3. Total biomass of caterpillars

There was a declining seasonal trend in total biomass during the nestling phase of great tit territories (log10-scale). Number of fledglings is back transformed from log10-scale.

### 3.4. Carotenoid concentration, food abundance and brood size in great tits

There was a declining seasonal trend in lutein concentration of nestlings’ plasma ($\chi^2 = 40.71, P < 0.0001$), lutein concentration being at its highest level in the end of May (Fig. 3). The number of fledglings was 27.7% higher in the polluted than in the polluted area ($\chi^2 = 10, P = 0.0016$). Mean number of fledglings was 4.08 (SD 1.68) in the polluted area and 5.64 (SD 1.52) in the unpolluted area. Early broods did not produce more fledglings than late broods ($\chi^2 = 0.87, P = 0.35$). Total larval biomass in great tit territories was positively correlated with the number of fledglings ($\chi^2 = 4.28, P = 0.039$, Fig. 4). The total biomass of larvae had no association...
4. Discussion

Although total caterpillar biomass increased during the breeding season, there was a simultaneous declining trend in lutein concentration of *P. major* nestling plasma both in the polluted and in the unpolluted area. Our result is most probably explained by the fact that although the total biomass of caterpillars increased in time due to increasing sawfly numbers, the biomass of the most lutein-rich caterpillars, autumnal moth larvae, decreased during the breeding season. In terms of total caterpillar biomass, food availability was higher for late than early great tit nestlings, but in terms of lutein availability, the food composition was better for early than late nestlings. This may result from seasonal changes in availability of different types of prey and their declining lutein concentration during the breeding season. The decreasing temporal trend in plasma lutein concentration is in contrast to the finding by Tummeleht et al. (2006), who found a positive temporal trend in adult breeding great tits. The possible difference between their and our study could be the differences in caterpillar species composition and in temporal availability among areas and habitats. The age and diet of the birds and spatial and temporal variations in prey availability could explain the difference in results of our study and the study of Tummeleht et al. (2006). Mols et al. (2005), found that the proportion of caterpillars in great tit nestling diet decreased with advancing date. Late nestlings may thus receive fewer caterpillars in their diet than early nestlings. The negative temporal trend in plasma lutein concentrations might have fitness consequences for nestlings of late broods, such as lowered immunocompetence (see e.g. Møller et al., 2000).

The negative association between caterpillar dry mass and lutein concentration may indicate that carotenoids, such as lutein, are probably depleted during larval development. Thus, in addition to the decreased proportion of lutein-rich caterpillars, late great tit nestlings may receive less lutein per unit mass of ingested caterpillar, although the body mass and total biomass of caterpillars increase during the breeding season. There may also be a temporal change in lutein concentration of birch leaves during the summer season, which reflect lutein reserves of herbivorous caterpillars. Unfortunately, no extensive data is available to assess the seasonal carotenoid concentration changes of birch leaves during summer, so this alternative remains to be studied. Sillanpää et al. (2008) studied the carotenoid concentration of birch leaves of downy birch (*Betula pubescens*) and silver birch (*Betula pendula*) from 1st June to 4th June, and Isaksson (2009) studied the carotenoid concentration of birch (*Betula verrucosa*) sun-shade leaves on 19th and 20th June. However, the species differ between the two studies and they do not cover the whole caterpillar periods of autumnal moths and sawflies. As a result, it is difficult to make general conclusions about the temporal changes in lutein concentration of birches and its effects on the lutein concentration of caterpillars.

The size (body mass) of autumnal moth and sawfly larvae did not differ between the polluted and the unpolluted area. However, the total biomass of all caterpillars was lower in the polluted area than in the unpolluted area. This may suggest that the great tit suffer from caterpillar deficiency in the diet in the polluted area (see also Eeva et al., 2005). Eeva et al. (2005) found that great tit took more beetles and other insects and less caterpillars of smaller size in the polluted area as compared to the unpolluted area. Note that the timing of the autumnal moth larval period and the amount of larvae is affected by pollution and may lead to a partial mismatch of peak larval abundance and great tit nestling demands in the polluted area. A mismatch between food supply and nestling demands likely entails fitness costs via reduced nesting growth and increased nesting mortality (Eeva and Lehikoinen, 1996; Thomas et al., 2001).

Tremblay et al. (2003) found that early breeding blue tits (*Cyanistes caeruleus*) are intrinsically different from late breeders and that this leads to larger and possibly to higher quality offspring produced by early breeders. This quality hypothesis also applies to the great tit (see Verhulst et al., 1995). Moreover, food requirements per nestling may differ between early and late broods (Mols et al., 2005), partly because food requirements declines as air temperature rises (Mertens, 1969). Selection should favour early clutches that coincides with the peak food availability (Garamszegi et al., 2004). In our study, early broods did not, however, differ in the number of fledglings compared to late broods and causal link between hatching date and fledgling survival has been suggested for tits (Verhulst and Tinbergen, 1991; Norris, 1993). The declining seasonal trend in lutein concentration of nestling plasma may suggest that early breeders are at an advantage compared to late breeders, when it comes to carotenoid availability. Since our study is based on correlative data, causality among different factors and the mechanisms behind them should be confirmed experimentally.

5. Conclusions

Different caterpillar species peak at different times during the nesting phase of the great tit and this may differently affect early and late broods, for example due to different carotenoid composition of caterpillars (see Sillanpää et al., 2008). Food availability should be better for late than early great tit nestlings, if total biomass of all caterpillars is concerned. On the other hand, in terms of lutein availability, food composition was better for early than late nestlings, which may result in decreased plasma lutein concentrations in late great tit nestlings. The seasonal decline of lutein concentration of late nestlings’ plasma may be explained by simultaneous decrease of biomass of lutein-rich autumnal moth larvae and also by the seasonal decline of lutein concentration in larvae. Although the biomass of sawfly larvae increased during the breeding season, their lutein concentration was relatively low. The ability of parents to provide their nestlings with a carotenoid-rich diet may enhance antioxidant protection, body condition, immunocompetence of offspring and thus overall fitness of nestlings (Biard et al., 2006; Koutsos et al., 2003). However, in addition to diet quality diet quantity also has important consequences: the larger the total biomass of larvae available during the nesting period, the higher the number of fledglings. The lower total biomass of caterpillars in the polluted area resulted in a lower number of fledglings as compared to the number in the unpolluted area.

Ethical standards

The study was performed under the licenses of the Animal care & use Committee of Turku University and Regional Environment Centre.

Conflict of interest

The authors declare that we have no conflict of interest.

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