Predator proximity as a stressor in breeding flycatchers: mass loss, stress protein induction, and elevated provisioning

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Abstract. We investigated the physiological and behavioral consequences for prey breeding at different distances from a nesting predator. In a natural setting, Pied Flycatchers (*Ficedula hypoleuca*) made territory location decisions relative to established Sparrowhawk (*Accipiter nisus*) nests. From female flycatchers attending nests at different distances from Sparrowhawk nests, we measured body mass, blood stress protein (HSP60 and HSP70), and plasma immunoglobulin levels at the beginning (initial) and end (final) of the flycatcher breeding cycle, and provisioning rates during the nestling phase. We found that individuals breeding in closer proximity to Sparrowhawk nests, under higher perceived predation risk, showed significantly lower body mass, higher stress protein and immunoglobulin levels, and higher nestling provisioning rates compared to those individuals breeding farther away. Across the range of distances investigated (30–610 m), final stress protein levels decreased linearly with distance, whereas the final measures of the other variables showed unimodal trends, increasing or decreasing until an intermediate distance (~350 m) and reversing the direction of the trend. Within 300 m, however, all measures showed significant linear associations with distance from the Sparrowhawk nest. Body mass and stress protein associations with distance from Sparrowhawk nests were only present during late breeding, and not in early incubation. Spatial proximity to Sparrowhawk nests consistently explained significant variation in both physiological and behavioral measures, despite the multitude of potential sources of variation for these measures in a natural setting. This suggests that predictable spatial patterns in these measures in avian communities are determined by the sites of breeding predators. Habitat selection decisions of migrant prey that vary only slightly spatially have consequences even at the cellular level, which plausibly have impacts on individual survival. In addition, this study suggests that predation risk is an important factor affecting physiological condition of prey, including stress protein induction in terrestrial vertebrates.

Key words: Accipiter nisus; anti-predator behavior; Ficedula hypoleuca; habitat selection; heat shock proteins; immunoglobulin; Pied Flycatcher; predation risk landscape; Sparrowhawk.

INTRODUCTION

Nonlethal impacts on prey stem from the direct fatal threat of predators. Behaviorally mediated impacts via the trade-off between foraging and antipredator behavior have received the most attention (Lima 1998). Increased perceived predation risk will heighten antipredator behaviors, resulting in reduced energy intake, which causes lower growth or maintenance, decreased reproductive success, and lower survival. However, nonlethal costs of predation risk may also stem from an underlying physiologically mediated component. Predation risk, via increased metabolic rates and the induction of stress compounds, may hinder energy metabolism and result in costs to individuals (Steiner 2007). Yet we lack an understanding of the ecologically relevant impacts of predation risk on behaviorally and physiologically induced reproductive outcomes.

Life history theory predicts that, during breeding, individuals should optimize energy investment between the current brood and self-maintenance. The outcome of this trade-off will depend on the value of the current brood and the residual reproductive value of the individual (Stearns 1992). Reproduction in birds is costly, and especially in short-lived altricial species, the value of the current brood will always be high (Magnhagen 1991). Additional costs stemming from breeding in poor habitat should lower the condition of parent birds and decrease the energy available to be

invested in either their current nestlings or future reproduction (Gustafsson et al. 1994, Moreno et al. 2002).

Recent studies have highlighted the use of cues that reliably indicate habitat quality (Doligez et al. 2002, Seppänen et al. 2007). Predation risk is a vital component of the habitat, especially during breeding. In birds, evidence shows that predator presence is assessed prior to settlement decisions (Fontaine and Martin 2006). During territory selection, birds avoid (Norrdahl and Korpimäki 1998) or are attracted to (Quinn and Ueta 2008) the vicinity of predator nest sites. By commuting to and from their nests, avian predators appear to anchor perceived predation risk gradients for their prey, coined a predation risk landscape (Thomson et al. 2006a, b).

Sparrowhawks (*Accipiter nisus*), the main predator of small adult Eurasian songbirds (Newton 1986), arrive in the north, initiate breeding, and are "waiting" in the landscape prior to the arrival of most migrant passerine species, including the Pied Flycatcher (*Ficedula hypoleuca*), hereafter "flycatcher." We previously established that flycatchers show fine-tuned territory location, preferring intermediate distances (~300–400 m from a hawk nest) where the costs of adult predation risk are assumed to be at a trade-off with protective benefits that the predators provide (Thomson et al. 2006b; see also Quinn and Kokorev 2002). In forests, predator encounters will be high near the predator nest but will decrease and be largely unchanging >300 m from the nest (Forsman et al. 2001). However, the physiological and behavioral implications of predator proximity to breeding birds are largely unknown.

We know that, to avoid predation, prey show behavioral and physiological changes (Cresswell 2008). The stress response probably forms an integral part of the nonlethal effects of predators on prey. This complex response is characterized by many components, one of which is the induction of stress proteins (so-called heat shock proteins, HSPs), which function in maintaining cellular homeostasis (Krebs and Loeschcke 1994). Stress protein induction relative to risk of predation has only recently been investigated under laboratory conditions in aquatic invertebrates (Pijanowska and Kloc 2004, Pauwels et al. 2005) and fish (Kagawa and Mugiya 2002). Increased exposure to predation risk resulted in higher levels of HSPs. Recent HSP research has focused on ecologically and evolutionary relevant stressors under natural environmental conditions (Sørensen et al. 2003) and may offer a more appropriate measure of long-term or chronic stress (Herring and Gawlik 2007). In natural bird populations, evidence of increased stress protein induction (HSP60 or HSP70) exists under nestling competition (Martínez-Padilla et al. 2004), higher parental effort (Merino et al. 2006), nutritional stress (Bourgeon et al. 2006), and parasitism (Merino et al. 1998, 2002, Tomás et al. 2005). Like parasitism,

predation risk is an important and ecologically relevant biotic stressor.

A further likely cost of HSP induction is the resulting down-regulation of the immune function (Morales et al. 2006). Immunoglobulin (IgY) levels may be used as a quantification of the humoral component of the immune system. IgY functions to intercept and bind to foreign bodies, such as blood parasites, and neutralize their effect. Due to the relationship between IgY and parasites, high levels of IgY may be an indication of a recent infection and, consequently, poor health (Morales et al. 2004). However, IgY levels may also be an indication of immune capacity and, taken together with other physiological aspects, may provide a measure of physiological costs.

In this study, we took a mechanistic approach to explore the behavioral and physiological consequences of habitat selection decisions of Pied Flycatchers relative to the spatial proximity of Sparrowhawk nests. We investigated the response of flycatcher body mass, heat shock protein level, immunoglobulin level, and nestling provisioning rates at distances up to 610 m from nesting Sparrowhawks. We also investigated responses only within 300 m from the nest, because Forsman et al. (2001) predicted that, due to the geometrically increasing area with linearly increasing distance from a hawk's nest, predation risk becomes practically unvarying after that threshold. If perceived predation risk gradients from breeding predators exist, we expect that flycatchers settling under higher predation risk closer to Sparrowhawk nests should show (1) lower body mass, (2) higher stress protein and immunoglobulin levels, and (3) lower provisioning rates (if predation risk interferes with foraging) compared to parents settling in areas of low predation risk.

Methods

Study site and setup

Active Sparrowhawk nests, three each in 2003 and 2004, were located in early spring in the forests near Oulu, northern Finland (65° N, 25°30′ E). Mixed forests with varying proportions of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and birch (*Betula* spp.) characterized these territories. Nest boxes for Pied Flycatchers were placed in grid format within Sparrowhawk territories. The distance between consecutive boxes was ~60–70 m in all directions and boxes were between 10 and 630 m from Sparrowhawk nests. The Pied Flycatcher is a small, cavity-nesting tropical migrant passerine (Lundberg and Alatalo 1992). We used 95 Pied Flycatcher nests in analyses that were not directly affected by predation (i.e., single-parent nests; for details, see Thomson et al. 2006b). They were divided between Sparrowhawk territories as shown in Table 1.

After flycatcher arrival, egg laying, clutch size, hatching, and resulting nest success were closely monitored. We used laying date (separately standardized for each year) as an indication of arrival date and, hence,

TABLE 1. The distribution of Pied Flycatcher (*Ficedula hypoleuca*) nests (*N*) between different years and Sparrowhawk territories in northern Finland.

Sparrowhawk territory	Year	*N*	Distance to Sparrowhawk nest (km)	
			Minimum	Maximum
Vitsasuo	2003	25	0.06	0.61
Navettakangas	2003	8	0.05	0.48
Alakanava	2003	23	0.09	0.60
Veitsisuo	2004	11	0.04	0.47
Korpi	2004	14	0.03	0.39
Navettasuo	2004	14	0.10	0.50
Total		95	0.03	0.61

parental quality (Lundberg and Alatalo 1992). Halfway through incubation (±6 days before hatching), female flycatchers were trapped on the nest. Blood samples were immediately taken from the brachial vein and collected using a capillary tube (maximum 100 μL). Females were then weighed to the nearest 0.1 g with a digital scale, their wing measurements were taken to the nearest 1 mm, and they were banded with a numbered metal ring. Females were then released back onto the nest. Later during breeding, when nestlings were 12 days old, females were again captured, blood was sampled, and body mass was measured. Initial and final samples were therefore taken ~18 days apart. If males were caught (only during nestling provisioning), we measured their body mass and wing length.

Provisioning rates

We measured flycatcher parental provisioning rates for an hour when nestlings were 7–8 days old using video cameras in bins placed a few meters from nests. The day before recordings, dummy bins were placed to habituate individuals to their presence. From recordings we determined the feeding rates (feeds/10 minutes) of both parents together and separately.

HSP estimation

In the field, blood samples were placed in a cool box below 15°C and were transported to the laboratory. Samples remained in the cool box for periods less than 8 h, during which time no significant changes in HSP60 or HSP70 blood protein levels occur (Tomás et al. 2004). In the laboratory, samples were centrifuged (2000*g* [=19 614 m/s^2] for 5 min, Eppendorf Microcentrifuge, Model 5415D, Eppendorf AG, Hamburg, Germany) and serum and cell fractions were separated and frozen for later analysis. Stress protein HSP60 and HSP70 levels were quantified from the cellular fraction of blood by means of Western-blot, using monoclonal antibodies anti-HSP70 and anti-HSP60 (clone BRM22 and LK2, respectively; Sigma, St. Louis, Missouri, USA) as primary antibodies, and a peroxidase-conjugated secondary antibody (Sigma, St. Louis, Missouri, USA). Protein bands were quantified using image analysis

software for Windows (Scion Corporation 2000). Immunoreactivities (arbitrary units) were obtained using the following formula: Immunoreactivity = area × mean intensity of the band. Details of the method are described in Merino et al. (1998) and Tomás et al. (2004).

Immunoglobulin Y estimation

Immunoglobulin levels were quantified from plasma samples by direct enzyme-linked immunosorbent assay (ELISA) using a polyclonal rabbit anti-chicken IgG conjugated with peroxidase (Sigma, St. Louis, Missouri, USA). Absorbances were measured using a plate spectrophotometer at λ = 405 nm. Details and validation of the method are described by Martínez et al. (2003).

Statistical analysis

We used mixed linear models (PROC MIXED, Enterprise Guide 4.1, SAS Institute 2006) to test the association of physiological and behavioral measures with distance (or squared distance) to Sparrowhawk nests (predation risk). Squared distance to hawk nest was included to check for nonlinear relationships, because flycatcher nest site selection relative to Sparrowhawk nests shows this pattern (Thomson et al. 2006*b*). Degrees of freedom were estimated by the Satterthwaite method (West et al. 2007). Data were analyzed at two scales, chiefly using flycatcher data from the full range of distances (up to 610 m) from Sparrowhawk nest, but also using data of nests within 300 m of the Sparrowhawk nest.

For all physiological variables (body mass, HSP60, HSP70, and IgY), our sampling presented us with an initial measure (collected from the female during incubation) and with a final measure (collected from the female during rearing of ~12-day-old chicks). For initial measures, in addition to distance variables, we included as continuous fixed variables the laying date of the first egg and the clutch size. Sparrowhawk territory and year were included as random effects, to control for the fact that the flycatcher boxes were available in six Sparrowhawk territories across two years. For body mass models, the individual's wing length was included to control for body size. In HSP and IgY models, residuals of a regression between female wing length and incubation body mass were included as a covariate (single measure of condition) and blot number as a class variable (stems from the Western-blot technique, where blots may show variation; Merino et al. 1998). These analyses allowed us to test the initial relationship of physiological measures with distance from Sparrowhawk nests. We also analyzed the final physiological measures, and for those individuals possible, the change in the measures across season (final minus initial). These models included the same terms, but number of chicks replaced the clutch size term, and residuals of a regression between female wing length against provi-

TABLE 2. Results of linear mixed models that examine physiological measures taken from female Pied Flycatchers during early incubation, during provisioning of 12-day-old nestlings, and the change in measures across this period.

Variables	Initial measures				Final measures				Change across season			
	$\beta \pm SE$	df	F	P	$\beta \pm SE$	df	F	P	$\beta \pm SE$	df	F	P
Body mass	$R^2 = 0.13$				$R^2 = 0.34$				$R^2 = 0.18$			
Distance	0.49 ± 0.5	1, 91	0.88	0.35	5.14 ± 2.05	1, 76.3	6.30	0.014	0.96 ± 0.51	1, 76.9	3.52	0.065
Distance²					-6.57 ± 3.35	1, 75.6	3.84	0.054				
Wing length	0.19 ± 0.1	1, 91	12.82	<0.001	0.17 ± 0.05	1, 74.3	13.91	<0.001	-0.04 ± 0.05	1, 76.3	0.54	0.47
HSP70 level	$R^2 = 0.57$				$R^2 = 0.52$				$R^2 = 0.40$			
Distance	-468 ± 801	1, 66	0.34	0.56	-2086 ± 931	1, 64.2	5.02	0.028	-1716 ± 886	1, 63.6	3.75	0.057
Clutch size	246 ± 147	1, 66	2.80	0.099								
Laying date					-80.6 ± 40.5	1, 41.8	3.97	0.053	-94.9 ± 38.0	1, 37	6.24	0.017
Condition					-220.1 ± 204	1, 65	1.16	0.28	85.6 ± 196	1, 62.7	0.19	0.66
Blot		8, 66	9.74	<0.001		8, 64.3	6.56	<0.001		8, 62.9	3.48	0.002
Immunoglobulin	$R^2 = 0.10$				$R^2 = 0.24$				$R^2 = 0.09$			
Distance	-2.9 ± 1.1	1, 74	7.05	0.01	-4.76 ± 1.29	1, 72.5	13.63	<0.001	-0.18 ± 0.28	1, 73.6	0.4	0.53
Distance²	4.3 ± 1.8	1, 74	5.44	0.02	6.76 ± 2.05	1, 72.6	10.85	0.002				
Laying date					-0.03 ± 0.01	1, 71.3	5.90	0.02	-0.03 ± 0.01	1, 47.1	6.19	0.02
Condition					0.17 ± 00.06	1, 72.6	7.16	0.01				

Notes: Dependent variables are body mass and levels of stress protein (HSP70) and serum immunoglobulin (IgY); independent variables are distance and squared distance to a Sparrowhawk nest, wing length, clutch size, laying date, condition, and blot. Blot is included in the analysis to control for variability between the different blots where samples are analyzed, which is inherent to the Western blot technique. Condition refers to the residuals of the linear regression of body mass against wing length. Territory and year are included as random effects (Z-score-derived P values > 0.25). Numerator and denominator degrees of freedom were estimated by the Satterthwaite method (West et al. 2007). R^2 values (the proportion of variability accounted for by the final model) were calculated as $1 - [\Sigma(y_i - \hat{y}_i)^2 / \Sigma(y_i - \tilde{Y})^2]$, where $\tilde{Y}$ represents the sample mean, $\hat{y}_i$ is the predicted value of the individual i, and y_i is the observed value of individual i.

sioning body mass were included in HSP and IgY models.

Total (parents combined) and sex-specific provisioning rates were analyzed with a model including distance (and squared distance) to hawk nest, laying date, chick number, chick age, and time of recording. Provisioning rates were only recorded for a subset of nests. For all models, sample sizes vary because we failed to catch all birds or because we collected insufficient blood. To simplify models, we used stepwise backward elimination of nonsignificant terms. Final model selections were subsequently checked using AIC values, which provided models that were biologically the same. Results are presented as means ± SE.

RESULTS

Parents from full range of nests 30–610 m from Sparrowhawk

Female body condition decreased across the breeding cycle from initial mass during mid-incubation to final mass during provisioning of 12-day-old chicks (initial: 15.1 ± 0.08 g, $n = 94$ female flycatchers; final: 12.6 ± 0.08 g, $n = 81$ females). Initial maternal body mass was not explained by distance to Sparrowhawk nest (Table 2). In contrast, maternal body mass during nestling provisioning was significantly explained by distance to Sparrowhawk nest (Table 2). Females nesting closer to Sparrowhawks showed lower final body mass than females nesting farther away (Fig. 1a). Across season, individual female flycatchers nesting close to Sparrow-

hawk nests showed a tendency of increased loss of body mass compared with individuals breeding farther away (Table 2). Male body mass (during nestling provisioning), controlled for body size ($n = 59$ males), did not differ with distance from Sparrowhawk nest ($F_{1,55} = 0.18$, $P = 0.67$).

Female blood levels of HSP70 decreased across the breeding cycle (incubation, 8763.44 ± 154.4 units, $n = 77$; provisioning, 7687.6 ± 158.3 units, $n = 77$). Final female HSP70 levels were significantly explained by distance to Sparrowhawk nest, whereas initial HSP70 levels were not (Table 2). During provisioning, females nesting in closer proximity to Sparrowhawk nests showed significantly higher levels of HSP70 than females nesting farther away (Fig. 1b). Therefore, across the season, female flycatchers breeding in closer proximity to Sparrowhawk nests retained higher or induced significantly more HSP70 than flycatchers breeding farther away. Neither initial nor final HSP60 levels were associated significantly with distance from Sparrowhawk nests.

Overall, female IgY levels did not differ across the breeding cycle (initial, 0.93 ± 0.04, $n = 77$; final, 1.06 ± 0.04, $n = 79$). Both initial and final (Fig. 1c) female IgY levels were explained by the distance to Sparrowhawk nest and the quadratic term of distance to Sparrowhawk nest (Table 2). IgY levels were highest in females breeding closest to Sparrowhawk nests, decreasing until ~360 m from the Sparrowhawk nest and then increasing at farther distances. Because initial and final IgY levels showed the same association with distance from
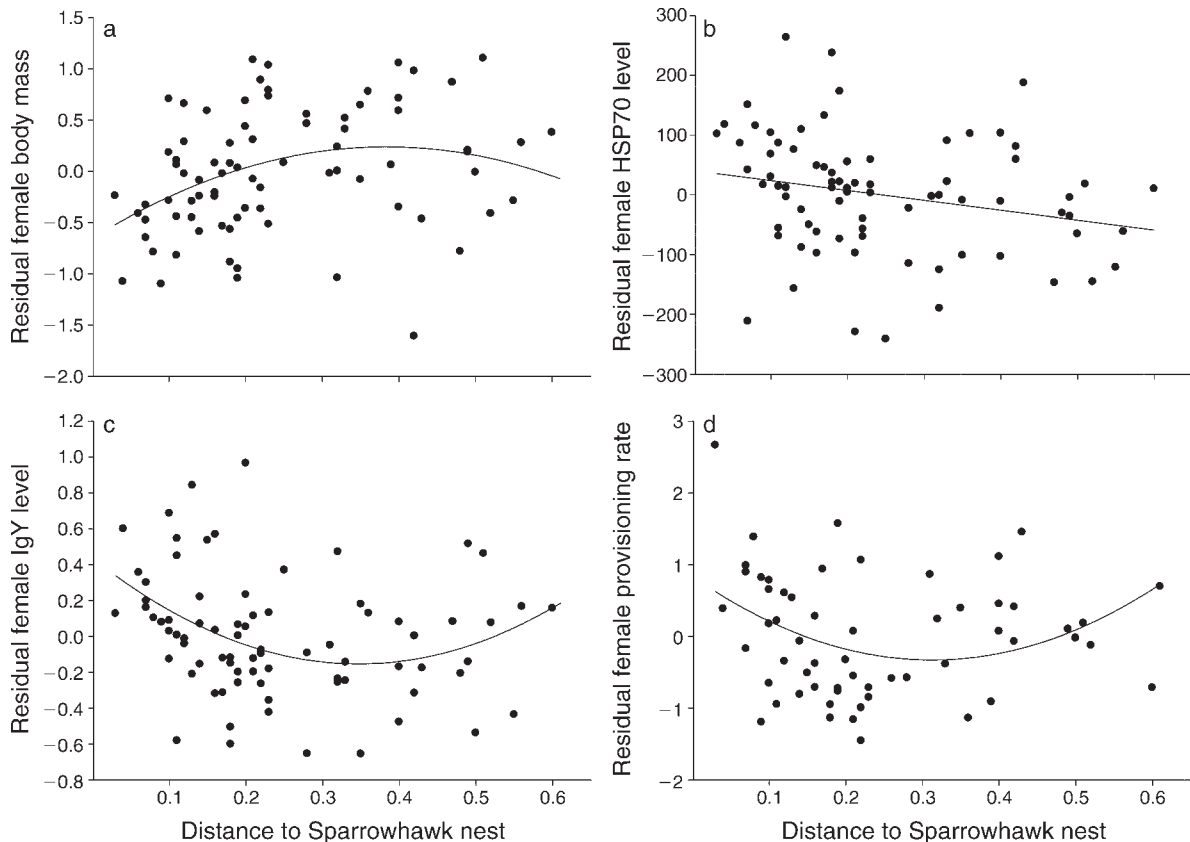
FIG. 1. Residual model values of the relationship between physiological and behavioral measures of female Pied Flycatchers (*Ficedula hypoleuca*) taken during nestling provisioning plotted against distance of their nest from a Sparrowhawk (*Accipiter nisus*) nest: (a) body mass controlled for body size, (b) stress protein (HSP70) levels, (c) serum immunoglobulin IgY levels, and (d) female feeding rates (feeds per chick per 10-minute observation).

Sparrowhawk nest, there was no across-season association with distance (Table 2).

Total provisioning rates were explained by chick number, distance to Sparrowhawk nests, and squared distance (Table 3; $n = 58$). Provisioning rates increased with the number of chicks in a nest. With chick number accounted for, flycatcher parents nesting in closer proximity to Sparrowhawk nests showed higher provisioning rates (Fig. 1d). Females were almost solely responsible for increased feeding rates closer to hawk nests (Table 3). Models for male provisioning were all nonsignificant ($P > 0.75$).

Females from nests < 300 m from the Sparrowhawk

At the smaller scale (only nests within 300 m), all final measures of variables taken during provisioning showed strong linear associations with distance from the hawk nest. Models were identical to those of final measures in Tables 2 and 3 except that the squared distance term dropped out in each case. Lower female body masses were found closer to the hawk nest, increasing linearly with distance ($R^2 = 0.49$; distance: $F_{1,49.3} = 15.23$, $P < 0.001$). Similarly, both HSP70 ($R^2 = 0.55$; distance: $F_{1,40.9} = 4.76$, $P = 0.035$) and immunoglobulin levels (R^2 $= 0.33$; distance: $F_{1,49.1} = 15.42$, $P < 0.001$) showed high levels close to hawk nests but decreased significantly linearly at distances farther from the hawk. Lastly, female provisioning rates ($R^2 = 0.43$; distance: $F_{1,36.1} =$

TABLE 3. Results of linear mixed models that examine provisioning rates during the flycatcher nesting period when nestlings were 7–8 days old.

Variables	β ± SE	df	F	P
Total provisioning rate ($R^2 = 0.34$)				
Distance	−12.56 ± 5.31	1, 53.9	5.60	0.02
Distance^2	17.58 ± 8.46	1, 53.5	4.32	0.04
Number of chicks	0.69 ± 0.21	1, 24.8	10.86	0.003
Female provisioning rate ($R^2 = 0.41$)				
Distance	−8.91 ± 3.29	1, 53.7	7.31	0.009
Distance^2	13.81 ± 5.24	1, 53.3	6.94	0.01
Number of chicks	0.51 ± 0.13	1, 33.8	14.53	<0.001

Notes: Territory and year were included as random effects and explained little variation (Z-score-derived P values > 0.1). Numerator and denominator degrees of freedom were estimated by the Satterthwaite method. R^2 values are calculated as $1 - [\Sigma(y_i - \hat{y}_i)^2/\Sigma(y_i - \tilde{Y})^2]$, where $\tilde{Y}$ represents the sample mean, $\hat{y}_i$ is the predicted value of the individual i, and y_i is the observed value of individual i.

TABLE 4. Absolute values of variables predicted from linear mixed-model regressions with distance from Sparrowhawk nests.

Variable	A) Nests from full range of distances			B) Nests closer than 300 m	
	30 m	Turning point	610 m	30 m	300 m
Body mass (g)	11.9	12.8 (at 391 m)	12.5	11.9	13.1
HSP70	8091	...	6906	8527	6917
IgY (absorbance)	1.56	0.86 (at 353 m)	1.31	1.54	0.65
Provisioning (feeds/10 min)	3.6	2.4 (at 322 m)	3.5	3.5	1.9

Notes: Values in (A) were predicted from models in Tables 2 and 3; values in (B) were predicted from models using only flycatcher nests within 300 m of the Sparrowhawk nest. Immunoreactivity of HSP70 is measured in arbitrary units, where immunoreactivity = area × mean intensity of the band (see Merino et al. 1998, Tomás et al. 2004).

7.74, $P < 0.01$) were high close to the hawk nest and decreased strongly with distance to 300 m from the hawk.

DISCUSSION

Physiological and behavioral measures of prey were consistently associated with distance from a breeding predator. It appears that Pied Flycatchers selecting territories close to Sparrowhawk nests were burdened with physiological costs during breeding. These costs included decreased body condition, higher stress protein and immunoglobulin Y levels, and altered work load, which all associated significantly with distance to the Sparrowhawk nest, despite the multitude of potential sources of variation for these measures in a natural setting.

Maternal body mass during provisioning showed a unimodal trend with distance from the predator nest, positively associated initially, reaching a peak at 390 m, and then decreasing slightly thereafter (Table 4). No association was found for incubation body mass. This suggests that additional costs are associated with breeding and raising chicks nearer to Sparrowhawk nests, although the change across season was not strictly significant. Within 300 m, female mass showed a strong positive association with distance from hawk nest; at 30 m, females were predicted to show body mass of 11.9 g increasing to 13.1 g at 300 m (Table 4), a change of about 10%. Under increased predation risk, birds generally show reduced body mass (Gentle and Gosler 2001) to improve escape possibilities due to mass-dependent performance (Cuthill and Houston 1997). In breeding birds, the sharp mass loss following egg hatching (Moreno 1989) is thought to be either for energy-efficient nestling provisioning or due to the increased physiological stress of provisioning (Hillström 1995, Merilä and Wiggins 1997). Our results suggest that mass loss accelerates due to increased costs of provisioning nestlings under high predation risk or maintaining high body mass under increased risk. We cannot exclude adaptive mass loss, but irrespective of the ultimate mechanism, our results suggest that females forfeit self-maintenance when they are closer to Sparrowhawk nests.

Maternal HSP70 during provisioning showed a negative association with distance from the Sparrow-

hawk nest, but no association ~18 days earlier for incubating females. Higher HSP70 levels were found in females breeding close to Sparrowhawks, with levels declining with distance from the predator nest. Within 300 m, this equates to 19% higher HSP70 levels at 30 m compared to 300 m from the hawk nest (Table 4). Stress protein induction relative to predation risk has earlier been shown in invertebrates and fish (Kagawa and Mugiya 2002, Pijanowska and Kloc 2004, Pauwels et al. 2005, Slos and Stoks 2008) but not in birds. Our results are particularly noteworthy because they demonstrate HSP induction in an ecologically natural setting (Sørensen et al. 2003, Herring and Gawlik 2007, Sørensen and Loeschcke 2007) where the spatial and temporal predation risk experienced by the individuals would show natural variation (Lima and Bednekoff 1999) and with many other potential stressors unrelated to distance from the Sparrowhawk nest. In birds, increased HSP levels have been shown in response to parasitism levels in naturally breeding populations (Merino et al. 1998, 2002, Tomás et al. 2005).

Immunoglobulin (IgY) levels both during incubation and nestling provisioning showed unimodal trends with distance from Sparrowhawk nests. Highest IgY levels were found in flycatchers nesting closest to the predator, reaching lowest levels around 350 m. If we considered only the closest 300 m, there was a sharp decline in IgY levels with distance, a difference of ~42% (Table 4). The exact interpretation of immunoglobulin levels remains problematic in avian ecology, as they may reflect the presence of an infection or immunocompetence (Norris and Evans 2000).

We found higher provisioning rates under increased predation risk closer to Sparrowhawk nests, with a sharp decline in provisioning toward 300 m from the predator. At distances farther than 300 m, the trend changed, resulting in a unimodal relationship with distance. Including only nests within 300 m, this amounts to a 54% decrease in feeding rates across this distance (Table 4). Increased provisioning rates under high predation risk present a surprising, but not entirely unexpected, result. Hakkarainen et al. (2002) reported 25% higher provisioning rates by Pied Flycatchers under perceived increased predation risk for adults and nests. An increasing frequency of feedings may stem from parents (1) trying to silence nestlings to decrease the

conspicuousness of the nest location, (2) attempting to advance fledging to reduce exposure to predation, and (3) remaining in the nest vicinity where they would be safer due to detailed knowledge of this area. Alternatively, (4) there may be less competition for food from other passerines closer to hawk nest. However, the smallest nestlings were produced in nests closest to Sparrowhawk nests (Thomson et al. [2006b]; partly the same nests as this study), which suggests that food quality or quantity were reduced despite increased delivery rates, and parents may invest less in nest sanitation, causing chicks to suffer from increased ectoparasite loads.

For the full range of distances, the unimodal relationships of body mass, IgY, and provisioning rate to distance from a hawk nest suggest less predictability in predation risk farther than 300 m from the hawk nest. This result matches an earlier prediction that hawk encounter rates would be largely unchanging after this distance (Forsman et al. 2001). Flycatchers are also known to prefer nest boxes at ~300 m from Sparrowhawk nests, where the costs (predation risk) and benefits (protection from other predators) are at a trade-off (Thomson et al. 2006b). Within 300 m, all measures show quite strong associations with distance to a predator nest. This simple landscape measure may provide unique potential for large-scale predictability of behavioral and physiological measures in prey individuals. We know of no other studies suggesting methods that would permit such an extrapolation of behavior or physiology at a natural landscape scale in a mobile vertebrate population. We suggest that, in avian communities, the sites of breeding avian predators gives rise to a "predation risk landscape" with the potential to explain significant variation in spatial settlement patterns (Thomson et al. 2006b, Mönkkönen et al. 2007), initial reproductive investment (Thomson et al. 2006b, Morosinotto et al. 2010), and also behavioral and physiological measures (this study). That the predators in our system are established prior to prey arrival probably enhances the predictability of these gradients to prey and to ecologists.

Our results for individual variables relative to increased predation risk are noteworthy. However, disentangling the interactions of our variables is problematic, especially for provisioning rate, because we cannot be sure that increased provisioning was indeed increased physical effort. Immunoglobulins are down-regulated by extra effort, which might suggest lower parental effort by flycatchers near Sparrowhawk nests. Moreno et al. (2002) found that maternal stress measures were related to increased provisioning rates at experimentally enlarged Pied Flycatcher broods, whereas Merino et al. (2006) found a reduction in stress protein levels in Blue Tits (*Parus caeruleus*) attending experimentally reduced broods. However, opposite to our findings, Blue Tits attending enlarged brood that needed higher provisioning showed lower immunoglob-

ulin levels (Merino et al. 2006). On the other hand, stress protein and plasma immunoglobulin levels have been shown to positively interact during offspring provisioning in another hole-nesting passerine (Merino et al. 2006). Both are probably costly to produce; hence increased levels in both suggest physiologically stressed individuals. Certain stress proteins play key roles in the modulation of the immune system (Feder and Hofmann 1999), but overall, much research is still needed before we fully understand the mechanisms through which stress protein and immunoglobulin levels may relate to each other (Merino et al. 2006).

Of importance for interpreting the results is that our field approach was correlative. Our analyses can statistically control for differences in individual quality by controlling for laying date (early arrivals are thought to be of highest quality), but biologically relevant individual quality trends with distance from hawk may, and probably do, remain. That initial measures of body mass and HSPs were unrelated to distance from hawk nest suggests that our results were not overly biased by this problem, although initial IgY levels could reflect the opposite. Indeed, high HSP levels during incubation (due to energetically demanding activities such as egg laying; Morales et al. 2004) may obscure any relationship with other stressors. This work should encourage experimental investigation into the predictability of behavioral and physiological measures in the landscape and the role of individual quality in these measures.

Our study suggests physiological costs/implications of breeding in high predation risk, with HSP induction in response to predation risk in an ecologically relevant setting. HSPs are produced gradually in response to extrinsic stress (Tomás et al. 2004) and require higher levels of stress to be elicited than do glucocorticoids (Herring and Gawlik 2007). HSPs may therefore provide an ideal measure of the stress response over longer time periods, such as a breeding season. Lack of HSP60 response in our study is similar to that found in previous studies of HSP induction in response to predation in other taxa (Slos and Stoks 2008). However, there is still a poor understanding of the precise conditions or stressors leading to the induction of either HSP60 or HSP70 (Morales et al. 2006, Herring and Gawlik 2007). Stress hormones, in contrast, induce rapidly for the short-term fight-or-flight response of animals. Glucocorticoid (stress) hormones have been used earlier to reflect the effects of environmental stress on adult birds (Silverin 1998, Cockrem and Silverin 2002, Suorsa et al. 2003) and even on nestlings (Blas et al. 2005). Scheuerlein et al. (2001) showed that Stonechats (*Saxicola torquatus*) breeding in shrike (*Lanius* sp.) territories maintained higher baseline levels of corticosterone. Further experimental studies will be required to fully understand the physiological costs of predation risk (Navarro et al. 2004). Our results show the need to combine physiological, ecological, and behavioral data to gain complete understanding of

community demographics in the evolution of life history strategies (Buchanan 2000).

In this study we have found that a small difference in spatial territory selection of a prey relative to a breeding predator can have quite large impacts at the behavioral and physiological level. This ultimately appears to translate into consequences for short-term reproductive output, but may also entail longer-term fitness costs through lower investment in self-maintenance and decreased probability of future reproduction. Uniquely, our study suggests that a migrant birds' habitat selection relative to the proximity of a breeding avian predator at the landscape scale may have relatively predictable physiological and behavioral implications, with plausible consequences for the survival of individuals.

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