1   Running head: Predator proximity as a stressor

2

3   Predator proximity as a stressor during breeding: increased mass loss,

4   stress protein induction and work load in female flycatchers

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1 *Abstract.* We investigated the physiological and behavioral consequences for prey breeding at

2 different distances to a nesting predator. In a natural setting, pied flycatchers (*Ficedula hypoleuca*)

3 made territory location decisions relative to established sparrowhawk (*Accipiter nisus*) nests. From

4 female flycatchers attending nests at different distances from sparrowhawk nests we measured

5 body mass, blood stress protein (HSP60 and HSP70) and plasma immunoglobulin levels at the

6 beginning (initial) and end (final) of the flycatcher breeding cycle, and provisioning rates during

7 the nestling phase. We found that individuals breeding in closer proximity to sparrowhawk nests,

8 under higher perceived predation risk, showed significantly lower body mass, higher stress protein

9 and immunoglobulin levels and higher nestling provisioning rates compared to those individuals

10 breeding further away. Across the range of distances investigated (30 m – 610m), final stress

11 protein levels decreased linearly with distance, while the final measures of the other variables

12 showed unimodal trends increasing or decreasing until an intermediate distance (~350 m) and

13 reversing the direction of the trend. Within 300 m however, all measures showed significant linear

14 associations with distance from sparrowhawk nest. Body mass and stress protein associations with

15 distance from sparrowhawk nests were only present later during breeding but not in early

16 incubation. Spatial proximity to sparrowhawk nests consistently explained significant variation in

17 both physiological and behavioral measures despite the multitude of potential sources of variation

18 for these measures in a natural setting. This suggests predictable spatial patterns in these measures

19 in avian communities determined by the sites of breeding predators. Habitat selection decisions of

20 migrant prey that spatially vary only slightly have consequences even at the cellular level, which

21 plausibly have impacts on individual survival. In addition, this study demonstrates that predation

22 risk is an important factor affecting prey physiological condition, including stress protein

23 induction in terrestrial vertebrates.

24

1 *Key words: heat shock proteins; immunoglobulin; predation risk landscape; trade-off; pied*

2 *flycatcher; sparrowhawk; anti-predator behavior; habitat selection*

3

4 INTRODUCTION

5 Non-lethal impacts on prey stem from the direct fatal threat of predators. Behaviorally mediated

6 impacts via the trade-off between foraging and anti-predator behavior have received the most

7 attention (Lima 1998). Increased perceived predation risk will heighten anti-predator behaviors

8 resulting in reduced energy intake causing lower growth or maintenance, decreased reproductive

9 success and lower survival. However, non-lethal costs of predation risk may also stem from an

10 underlying physiologically mediated component. Predation risk, via increased metabolic rates and

11 the induction of stress compounds may hinder energy metabolism and result in costs to individuals

12 (Steiner 2007). But we lack an understanding of ecologically relevant impacts of predation risk on

13 behaviorally and physiologically induced reproductive outcomes.

14

15 Life history theory predicts that during breeding, individuals should optimise energy investment

16 between the current brood and self-maintenance. The outcome of this trade-off will depend on the

17 value of the current brood and the residual reproductive value of the individual (Stearns 1992).

18 Reproduction in birds is costly, and especially in short-lived altricial species, the value of current

19 brood will always be high (Magnhagen 1991). Additional costs stemming from breeding in poor

20 habitat should lower the condition of parent birds and decrease the energy available to be invested

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

22

23 Recent studies have highlighted the use of cues that reliably indicate habitat quality (Doligez et al.

24 2002; Seppänen et al. 2007). Predation risk is a vital component of the habitat, especially during

1   breeding. In birds, evidence shows that predator presence is assessed prior to settlement decisions

2   (Fontaine and Martin 2006). During territory selection birds avoid (Norrdahl and Korpimäki 1998)

3   or are attracted to (Quinn and Ueta 2008) the vicinity of predator nest sites. By commuting to and

4   from their nests, avian predators appear to anchor perceived predation risk gradients for their prey

5   coined a predation risk landscape (Thomson et al. 2006a, b).

6

7   Sparrowhawk (*Accipiter nisus*), the main predator of small adult songbirds (Newton 1986), arrive

8   in the north, initiate breeding, and are "waiting" in the landscape prior to the arrival of most

9   migrant passerine species, including pied flycatcher (*Ficedula hypoleuca*). We previously

10   established that flycatchers show fine-tuned territory location, preferring intermediate distances (c.

11   300 – 400 m from a hawk nest) where adult predation risk is assumed to be at a trade-off with

12   protective benefits that the predators provide (Thomson et al. 2006b, see also Quinn and Kokorev

13   2002). In forests, predator encounters will be high near the predator nest but will decrease and be

14   largely unchanging >300m from the nest (Forsman et al. 2001). However, the physiological and

15   behavioral implications of predator proximity to breeding birds are largely unknown.

16

17   We know that to avoid predation prey show behavioral and physiological changes (e.g. Cresswell

18   2008). The stress response likely forms an integral part of the non-lethal effects of predators on

19   prey. This complex response is characterized by many components, one of which is the induction

20   of stress proteins (also called heat shock proteins; HSPs), which function in maintaining cellular

21   homeostasis (Krebs and Loeschcke 1994). Stress protein induction relative to risk of predation has

22   only recently been investigated under laboratory conditions in aquatic invertebrates (Pijanowska

23   and Kloc 2004, Pauwels et al. 2005) and fish (Kagawa and Mugiya 2002). Increased exposure to

24   predation risk resulted in higher levels of HSPs. Recent HSP research has focussed on ecologically

1   and evolutionary relevant stressors under natural environmental conditions (Sørensen et al. 2003)

2   and may offer a more appropriate measure of long-term or chronic stress (Herring and Gawlik

3   2007). In natural bird populations, evidence of increased stress protein induction (HSP60 and

4   HSP70) exists under nestling competition (Martínez-Padilla et al. 2004), higher parental effort

5   (Merino et al. 2006), nutritional stress (Bourgeon et al. 2006) and parasitism (Merino et al. 1998,

6   Merino et al. 2002, Tomás et al. 2005). Like parasitism, predation risk is an important and

7   ecologically relevant biotic stressor.

8

9   A further likely cost of HSP induction is the resulting down regulation of the immune function

10   (Morales et al. 2006). Immunoglobulin (IgY) levels may be used as a quantification of the humoral

11   component of the immune system. IgY functions to intercept and bind to foreign bodies, such as

12   blood parasites, and neutralise their effect. Due to the relationship between IgY and parasites, high

13   levels of IgY may be an indication of a recent infection and consequently poor health (Morales et

14   al. 2004). However, IgY levels may also be an indication of immune capacity and taken together

15   with other physiological aspects may provide a measure of physiological costs.

16

17   In this study, we take a mechanistic approach to explore the behavioral and physiological

18   consequences of habitat selection decisions of pied flycatchers relative to spatial proximity of

19   sparrowhawk nests. We investigate the response of flycatcher body mass, heat shock protein level,

20   immunoglobulin level and nestling provisioning rates at distances up to 610m from nesting

21   sparrowhawks. We also investigated responses only within 300 m from the nest because Forsman

22   et al. (2001) predicted that due to the geometrically increasing area with linearly increasing

23   distance from a hawk's nest, predation risk becomes practically unvarying after that threshold. If

24   perceived predation risk gradients from breeding predators exist, we expect flycatchers settling

1   under higher predation risk closer to sparrowhawk nests should show a) lower body mass , b)

2   higher stress protein and immunoglobulin levels and c) lower provisioning rates (if predation risk

3   interferes with foraging) compared to parents settling in low predation risk areas.

4

5   METHODS

6   **Study site and set-up**

7   Active sparrowhawk nests, three each in 2003 and 2004, were located in early spring in the forests

8   near Oulu, northern Finland (65° N, 25°30'E). Mixed forests with varying proportions of Scots

9   pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and birch (*Betula* spp.) characterised these

10   territories. Nest boxes for pied flycatchers were placed in grid format within sparrowhawk

11   territories. The distance between consecutive boxes was roughly 60 - 70 m in all directions and

12   boxes were between 10 - 630 m from sparrowhawk nests. The pied flycatcher is a small cavity

13   nesting tropical migrant passerine (Lundberg an Alatalo 1992). We used 95 pied flycatcher nests

14   in analyses that were not directly affected by predation (see Thomson et al. 2006b for details).

15   They were divided between sparrowhawk territories as shown in Table 1.

16

17   After flycatcher arrival, egg-laying, clutch size, hatching and resulting nest success were closely

18   monitored. We used laying date (separately standardized for each year) as an indication of arrival

19   date and hence parental quality (Lundberg and Alatalo 1992). Halfway through incubation (± 6

20   days before hatching) female flycatchers were trapped on the nest. Blood samples were

21   immediately taken from the brachial vein and collected using a capillary tube (max. 100 µl).

22   Females were then weighed to the nearest 0.1 g with a digital scale, their wing measurements

23   taken to the nearest 1 mm and ringed with a numbered metal ring. Females were then released

24   back onto the nest. Later during breeding, when nestlings were 12 days old, females were again

1   captured, blood was sampled and body mass taken. Initial and final samples were therefore taken

2   ~18 days apart. If males were caught (only during nestling provisioning), we measured their body

3   mass and wing length. If only one adult bird was repeatedly observed at a nest, the nest was

4   classified as a single parent nest. Data from these nests were excluded from analyses.

5

6   **Provisioning rates**

7   We measured flycatcher parental provisioning rates for an hour when nestlings were 7 - 8 days old

8   using video cameras in bins placed a few meters from nests. The day before recordings, dummy

9   bins were placed to habituate individuals to their presence. From recordings we determined the

10   feeding rates (feeds/ 10 minutes) of both parents together and separately.

11

12   **HSP estimation**

13   In the field, blood samples were placed in a cool box below 15 ºC and transported to the

14   laboratory. Samples remained in the cool box for periods less than eight hours, during which time

15   no significant changes in HSP60 or HSP70 blood protein levels occur (Tomás et al. 2004). In the

16   laboratory, samples were centrifuged (2000 g for 5 min, Eppendorf Microcentrifuge, Model

17   5415D, Eppendorf AG, Hamburg, Germany) and serum and cell fractions were separated and

18   frozen for later analysis. Stress protein HSP60 and HSP70 levels were quantified from the cellular

19   fraction of blood by means of Western-blot, using monoclonal antibodies anti-HSP70 and anti-

20   HSP60 (clone BRM22 and LK2 respectively, Sigma, St.Louis, MO, USA) as primary antibodies,

21   and a peroxidase-conjugated secondary antibody (Sigma, St. Louis, MO, USA). Protein bands

22   were quantified using image analysis software for windows (Scion Corporation, Frederick, MD,

23   USA). Immunoreactivities (arbitrary units) were obtained using the following formula:

1   Immunoreactivity = area x mean intensity of the band. Details of the method are described in

2   Merino et al. (1998) and Tomás et al. (2004).

3

4   **Immunoglobulin Y estimation**

5   Immunoglobulin levels were quantified from plasma samples by direct enzyme-linked

6   immunosorbent assay (ELISA) using a polyclonal rabbit anti-chicken IgG conjugated with

7   peroxidase (Sigma, St.Louis, MO, USA). Absorbances were measured using a plate

8   spectrophotometer at λ= 405 nm. Details and validation of the method are described by Martínez

9   et al. (2003).

10

11 **Statistical analysis**

12 We used mixed linear models (PROC MIXED, SAS Enterprise Guide 4) to test the association of

13 physiological and behavioral measures with distance (or squared distance) to sparrowhawk nests

14 (predation risk). Squared distance to hawk nest was included to check for non-linear relationships

15 because flycatcher nest site selection relative to sparrowhawk nests shows this pattern (Thomson

16 et al. 2006b). Data was analyzed at two scales, chiefly using flycatcher data from the full range of

17 distances (up to 610 m) from sparrowhawk nest, but also using data of nests within 300 m of the

18 sparrowhawk.

19

20 For all physiological variables (body mass, HSP60, HSP70 and IgY) our sampling presented us

21 with an initial measure (collected from female during incubation) and with a final measure

22 (collected from female during rearing ~12 day old chicks), which we analyzed separately. For

23 initial measures, in addition to distance variables, we included as continuous fixed variables:

24 laying date of first egg and clutch size, and class variable year (2003 or 2004). Sparrowhawk

1 territory was included as a random effect, to control for the fact that the flycatcher boxes were

2 available in six sparrowhawk territories. For body mass models, the individual's wing length was

3 included to control for body size. In HSP and IgY models, residuals of a female incubation body

4 mass and wing length regression was included as a covariate (single measure of condition) and

5 blot number as a class variable (stems from the Western blot technique, where blots may show

6 variation due to unknown factors, Merino et al. 1998). These analyses allowed us to test initial

7 relationship of physiological measures with distance from sparrowhawk nests. We also analyzed

8 the final physiological measures and for those individuals possible, the change in the measures

9 across season (final minus initial value). These models included the same terms, however, number

10 of chicks replaced the clutch size term, and residuals of regression of female provisioning body

11 mass against wing length were included in HSP and IgY models.

12

13 Total (parents combined) and sex specific provisioning rates were analyzed with a model

14 including distance (and squared distance) to hawk nest, laying date, chick number, chick age and

15 time of recording. Provisioning rates were only recorded for a subset of nests. For all models,

16 sample sizes vary because we failed to catch all birds or insufficient blood was collected. To

17 simplify models, we used stepwise backward elimination of non-significant terms. Final model

18 selections were subsequently checked using AIC values, which provided biologically the same

19 results.

20

21 RESUTS

22 *Parents from full range of nests 30 m – 610 m from sparrowhawk*

23 Female body condition decreased across the breeding cycle from initial mass during mid-

24 incubation to final mass during provisioning of 12 day old chicks (mean ±SE; initial: 15.1 g ±0.08,

1   n = 94; final: 12.6 g ±0.08, n = 81). Initial maternal body mass was not explained by distance to

2   sparrowhawk nest (Table 2). In contrast, maternal body mass during nestling provisioning was

3   significantly explained by distance to sparrowhawk nest (Table 2). Females nesting closer to

4   sparrowhawks showed lower final body mass than females nesting further away (Fig. 1a). Across

5   season, individual female flycatchers nesting close to sparrowhawk nests showed a tendency of

6   increased body mass loss compared with individuals breeding further away. Male body mass

7   (during nestling provisioning), controlled for body size (n = 59), did not differ with distance from

8   sparrowhawk nest ($F_{1,55}$ = 0.18, p = 0.67).

9

10   Female blood levels of HSP70 decreased across the breeding cycle (incubation 8763.44 units

11   ±154.4, n = 77, provisioning 7687.6 units ±158.3, n = 77). Final female HSP70 levels were

12   significantly explained by distance to sparrowhawk nest, whereas initial HSP70 levels were not

13   (Table 2). During provisioning females nesting in closer proximity to sparrowhawk nests showing

14   significantly higher levels of HSP70 than females nesting further away (Fig. 1d). Therefore across

15   the season, female flycatchers breeding in closer proximity to sparrowhawk nests retained higher

16   or induced significantly more HSP70 than flycatchers breeding further away. Neither initial nor

17   final HSP60 levels associated significantly with distance from sparrowhawk nests.

18

19   Overall female IgY levels did not differ across the breeding cycle (initial: 0.93 ±0.04, n = 77; final:

20   1.06 ±0.04, n = 79). Both initial and final (Fig. 1c) female IgY levels were explained by the

21   distance to sparrowhawk nest and the quadratic term of distance to sparrowhawk nest (Table 2).

22   IgY levels were highest in females breeding closest to sparrowhawk nests, decreasing until

23   approximately 360m from the sparrowhawk nest and then increasing at further distances. Because

1   initial and final IgY levels showed the same association with distance from sparrowhawk nest

2   there was no across season association with distance (Table 2).

3

4   Total provisioning rates were explained by chick number, distance to sparrowhawk nests and

5   squared distance (Table 3, n = 58). Provisioning rates increased with the number of chicks in a

6   nest. With chick number accounted for, flycatcher parents nesting in closer proximity to

7   sparrowhawk nests showed higher provisioning rates (Fig 1b). Females were almost solely

8   responsible for increased feeding rates closer to hawk nests (Table 3). Models for male

9   provisioning were all non-significant (P > 0.75).

10

11   *Females from nests < 300 m from the sparrowhawk*

12   At the smaller scale (only nests within 300 m) all variables taken during provisioning (final

13   measures) showed strong linear associations with distance from the hawk nest. Final models were

14   identical to those in Table 2 & 3 except that the squared distance term dropped out in each case.

15   Lower female body masses were found closer to the hawk nest increasing linearly with distance

16   ($R^2 = 0.49$, distance: $F_{1,49.3} = 15.23$, p <0.001). Similarly, both HSP70 ($R^2 = 0.55$, distance: $F_{1,40.9}$

17   = 4.76, p = 0.035) and immunoglobulin levels ($R^2 = 0.33$, distance: $F_{1,49.1} = 15.42$, p < 0.001)

18   showed high levels close to hawk nests but decreased significantly linearly at distances further

19   from the hawk. Lastly, female provisioning rates ($R^2 = 0.43$, distance: $F_{1,36.1} = 7.74$, p < 0.01) were

20   high close to hawk nest decreasing quite substantially with distance to 300 m from the hawk.

21

22   DISCUSSION

23   Physiological and behavioral measures of prey were consistently associated with distance from a

24   breeding predator. It appears that pied flycatchers selecting territories close to sparrowhawk nests

1   were burdened with physiological costs during breeding. These costs included decreased body

2   condition, higher stress protein and immunoglobulin Y levels, and altered work load that all

3   associated significantly with distance to the sparrowhawk nest, despite the multitude of potential

4   sources of variation for these measures in a natural setting.

5

6   Maternal body mass during provisioning showed a unimodal trend with distance from the predator

7   nest, positively associated initially reaching a peak at 390 m and decreasing slightly thereafter

8   (Table 5). No association was found for incubation body mass. This suggests additional costs

9   associated with breeding and raising chicks nearer to sparrowhawk nests although the change

10 across season was not strictly significant. Within 300 m female mass showed a strong positive

11 association with distance from hawk nest, at 30m females were predicted to show body mass of

12 11.9 g increasing to 13.1 g at 300 m (Table 5), a change of about 10%. Under increased predation

13 risk, birds generally show reduced body mass (Gentle and Gosler 2001) to improve escape

14 possibilities due to mass-dependent performance (Cuthill and Houston 1997). In breeding birds,

15 the sharp mass loss following egg hatching (Moreno 1989) is thought to be either for energy

16 efficient nestling provisioning or due to the increased physiological stress of provisioning

17 (Hillström 1995, Merilä and Wiggins 1997). Our results suggest that mass loss accelerates due to

18 increased costs of provisioning nestlings in high predation risk or maintaining high body mass

19 under increased risk. We cannot exclude adaptive mass loss, but irrespective of the ultimate

20 mechanism, our results suggest that females forfeit self-maintenance closer to sparrowhawk nests.

21

22 Maternal HSP70 during provisioning showed a negative association with distance from

23 sparrowhawk nest with no association ~18 days earlier for incubating females. Higher HSP70

24 levels were found in females breeding close to sparrowhawks with levels declining with distance

1 from the predator nest. Within 300 m this equates to 19% higher HPP70 levels at 30 m compared

2 to 300 m from the hawk nest (Table 4). Stress protein induction relative to predation risk has

3 earlier been shown in invertebrates and fish (Kagawa and Mugiya 2002, Pijanowska and Kloc

4 2004, Pauwels et al. 2005, Slos and Stoks 2008) but not in birds. Our results are particularly

5 noteworthy because they demonstrate HSP induction in an ecologically natural setting (Sørensen

6 et al. 2003, Herring and Gawlik 2007, Sørensen and Loeschke 2007) where the spatial and

7 temporal predation risk experienced by the individuals would show natural variation (Lima and

8 Bednekoff 1999) and with many other potential stressors unrelated to distance from the

9 sparrowhawk nest. In birds, increased HSP levels have been shown in response to parasitism levels

10 in naturally breeding populations (Merino et al. 1998, Merino et al. 2002, Tomás et al. 2005).

11

12 Immunoglobulin (IgY) levels both during incubation and nestling provisioning showed unimodal

13 trends with distance from sparrowhawk nests. Highest IgY levels were found in flycatchers nesting

14 closest to the predator reaching lowest levels around 350 m. If only the closest 300m was

15 considered there was a sharp decline in IgY levels with distance, a difference of about 42% (Table

16 4). The exact interpretation of immunoglobulin levels remains problematic in avian ecology as

17 they may reflect the presence of an infection or immunocompetence (Norris and Evans 2000). Our

18 results likely indicate some systematic change of individual quality with distance.

19

20 We found higher provisioning rates under increased predation risk, closer to sparrowhawk nests

21 with a sharp decline in provisioning towards 300m from the predator. At distances further than

22 300m the trend changed resulting in a unimodal relationship with distance. Including only nests

23 within 300 m, this amounts to a 54% decrease in feeding rates across this distance (Table 4).

24 Increased provisioning rates in high predation risk present a surprising, but not entirely unexpected

1 result. Hakkarainen and co-authors (2002) reported 25% higher provisioning rates by pied

2 flycatchers under increased perceived adult and nest predation risk. An increasing frequency of

3 feeds may stem from parents i) trying to silence nestlings to decrease the conspicuousness of the

4 nest location, ii) attempting to advance fledging to reduce exposure to predation, iii) remaining in

5 the nest vicinity where they would be safer due to detailed knowledge of this area or alternatively

6 iv) there may be less competition for food from other passerines closer to hawk nest. However, the

7 smallest nestlings were produced in nests closest to sparrowhawk (Thomson et al. 2006b, partly

8 the same nests as this study) which suggests food quality or quantity were reduced despite

9 increased delivery rates and parents may invest less in nest sanitation causing chicks to suffer from

10 increased ectoparasite loads.

11

12 For the full range of distances, the unimodal relationships of body mass, IgY and provisioning rate

13 with distance from hawk nest suggest less predictability in predation risk further than 300m from

14 the hawk nest. This result matches an earlier prediction that hawk encounter rates would be largely

15 unchanging after this distance (Forsman et al. 2001). Flycatchers are also known to prefer nest

16 boxes at approximately 300m from sparrowhawk nests, where the costs (predation risk) and

17 benefits (protection from other predators) are at a trade-off (Thomson et al 2006b). Within 300 m

18 all measures show quite strong associations with distance to a predator nest. This simple landscape

19 measure may provide unique potential for large-scale predictability of behavioral and

20 physiological measures in prey individuals. We know of no other studies suggesting methods that

21 would permit such an extrapolation of behavior or physiology at a natural landscape scale in a

22 mobile vertebrate population. We suggest that in avian communities, the sites of breeding avian

23 predators gives rise to a "predation risk landscape" with the potential to explain significant

24 variation in spatial settlement patterns (Thomson et al. 2006b, Mönkkönen et al. 2007), initial

1   reproductive investment (Thomson et al. 2006b, Morosinotto, Thomson and Korpimäki

2   unpublished manuscript) but also behavioral and physiological measures (this study). That the

3   predators in our system are established prior to prey arrival likely enhances the predictability of

4   these gradients to prey (and ecologists).

5

6   Our results for individual variables relative to increased predation risk are noteworthy. However,

7   disentangling the interactions of our variables is problematic. Provisioning rate in particular

8   because we cannot be sure that increased provisioning was indeed increased physical effort.

9   Immunoglobulins are down-regulated by extra effort which might suggest lower parental effort by

10   flycatchers near to sparrowhawk nests. Moreno et al. (2002) found that maternal stress measures

11   were related to increased provisioning rates at experimentally enlarged broods, however opposite

12   to our findings, blue tits attending enlarged brood needing higher provisioning showed lower

13   immunoglobulin levels (Merino et al. 2006). Stress protein and plasma immunoglobulin levels

14   have been shown to positively interact during offspring provisioning in another hole-nesting

15   passerine (Merino et al. 2006). Both are likely costly to produce and increased levels in both

16   suggest physiologically stressed individuals.

17

18   Of importance for interpreting the results is that our field approach was correlative. Our analyses

19   can statistically control for differences in individual quality by controlling for laying date (early

20   arrivals are thought to be of highest quality), but biologically relevant individual quality trends

21   with distance form hawk may, and probably do remain. That initial measures of body mass and

22   HSPs were unrelated to distance from hawk nest suggests our results were not overly biased by

23   this problem, although initial IgY levels could reflect the opposite. Indeed, high HSP levels during

24   incubation (due to energetic demanding activities like egg laying, Morales et al. 2004) may

1   obscure any relationship with other stressors. This work should encourage experimental

2   investigation into the predictability of behavioural and physiological measures in the landscape

3   and the role of individual quality in these measures.

4

5   Our study suggests physiological costs/implications of breeding in high predation risk, with HSP

6   induction in response to predation risk in an ecologically relevant setting. HSPs are produced

7   gradually in response to extrinsic stress (Tomás et al. 2004) and require higher levels of stress to

8   be elicited than glucocorticoids (Herring and Gawlik 2007). HSPs may therefore provide an ideal

9   measure of the stress response over longer time periods such as a breeding season. Lack of HSP60

10   response in our study is similar to that found in previous studies of HSP induction in response to

11   predation in other taxa (Slos and Stoks 2008). However, there is still a poor understanding of the

12   precise conditions or stressors leading to the induction of either HSP60 or HSP70 (Morales et al.

13   2006, Herring and Gawlik 2007). Stress hormones, in contrast, induce rapidly for the short-term

14   fight-or-flight response of animals. Glucocorticoid (stress) hormones have earlier been used to

15   reflect the effects of environmental stress on adult birds (Silverin 1998, Cockrem and Silverin

16   2002, Suorsa et al. 2003) and even in nestlings (Blas et al. 2005). Scheuerlein et al. (2001) showed

17   that stonechats breeding in shrike territories maintained higher baseline levels of corticosterone.

18   Further experimental studies will be required to fully understand the physiological costs of

19   predation risk (Navarro et al. 2004). Our results show the need to combine physiological,

20   ecological and behavioral data to gain complete understanding of community demographics in the

21   evolution of life history strategies (Buchanan 2000).

22

23   In this study we have found that a small difference in spatial territory selection of a prey relative to

24   a breeding predator can have quite large impacts at the behavioral and physiological level. This

1 ultimately appears to translate into consequences for short-term reproductive output but may also

2 entail longer-term fitness costs through lower investment in self-maintenance and decreased

3 probability of future reproduction. Uniquely our study suggests that a migrant birds' habitat

4 selection relative to the proximity of breeding avian predator at the landscape scale may have

5 relatively predictable physiological and behavioral implications which plausibly have

6 consequences for the survival of individuals.

7

17

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1   Table 1. The distribution of pied flycatcher (*Ficedula hypoleuca*) nests between different years

2   and sparrowhawk territories. Distances are in kilometres.

Territory	Year	N	Minimum distance	Maximum distance
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

3

1 Table 2. Results of linear mixed models that examine physiological measures taken from females pied flycatchers during early

2 incubation, during provisioning of 12 day old nestlings and the change in measures across this period. Territory and year are included as

3 random effects (Z-score derived p-values > 0.25). Condition refers to the residuals of the linear regression of body mass against wing

4 length. Degrees of freedom represents numerator, denominator (estimated by the Satterthwaite method).

Independent	Dependent variable										
variable		*Initial measures*			*Final measures*				*Change across season*			
		Df	*F*	P	β (±SE)	df	*F*	P	β (±SE)	df	*F*	P
.	*Body mass*											
		$R^2 = 0.13^a$			$R^2 = 0.34^a$				$R^2 = 0.18^a$			
Distance		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
Distance^2					-6.57 (±3.35)	1,75.6	3.84	0.054				
Wing length		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											
		$R^2 = 0.57^a$			$R^2 = 0.52^a$				$R^2 = 0.40^a$			
Distance		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		1,66	2.80	0.099								

	Estimate	df	F	p	Estimate	df	F	p	Estimate	df	F	p
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 (IgY) level												
		$R^2 = 0.10^a$				$R^2 = 0.24^a$				$R^2 = 0.09^a$		
Distance		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
Distance^2		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 (±0.06)	1,72.6	7.16	0.01				

1 [a]Calculated as $1 - (\sum(y_i - \hat{y}_i)^2 / \sum(y_i - \tilde{Y})^2)$, where $\tilde{Y}$ represents the sample mean; $\hat{y}_i$ predicted value of the individual i; y_i the observed

2 value of individual i.

1   Table 3. Results of linear mixed models that examine provisioning rates during the nesting period

2   when nestlings were 7-8 days old. Territory and year were included as a random effects and

3   explained little variation (Z-score derived p-values > 0.1). Degrees of freedom represents

4   numerator, denominator (estimated by the Satterthwaite method).

Independent variable	Dependent variable			
	β ($\pm$se)	df	F-value	P
Total provisioning rate ($R^2 = 0.34$) [a]				
Distance	-12.56 ($\pm$5.31)	1,53.9	5.60	0.02
Distance^2	17.58 ($\pm$8.46)	1,53.5	4.32	0.04
Number of chicks	0.69 ($\pm$0.21)	1,24.8	10.86	0.003
Female provisioning rate ($R^2 = 0.41$) [a]				
Distance	-8.91 ($\pm$3.29)	1,53.7	7.31	0.009
Distance^2	13.81 ($\pm$5.24)	1,53.3	6.94	0.01
Number of chicks	0.51 ($\pm$0.13)	1,33.8	14.53	<0.001

5   [a] Calculated as $1 - (\sum(y_i - \hat{y}_i)^2/\sum(y_i - \tilde{Y})^2)$, where $\tilde{Y}$ represents the sample mean; $\hat{y}_i$ predicted value

6   of the individual *i*; y_i the observed value of individual *i*.

1   Table 4: Absolute values of variables predicted from linear mixed model regressions with distance

2   from sparrowhawk nests. A: predicted from models in Table 2 and 3, B: predicted from models

3   using only nests within 300 m of the sparrowhawk nest. HSP70 and IgY use arbitrary units.

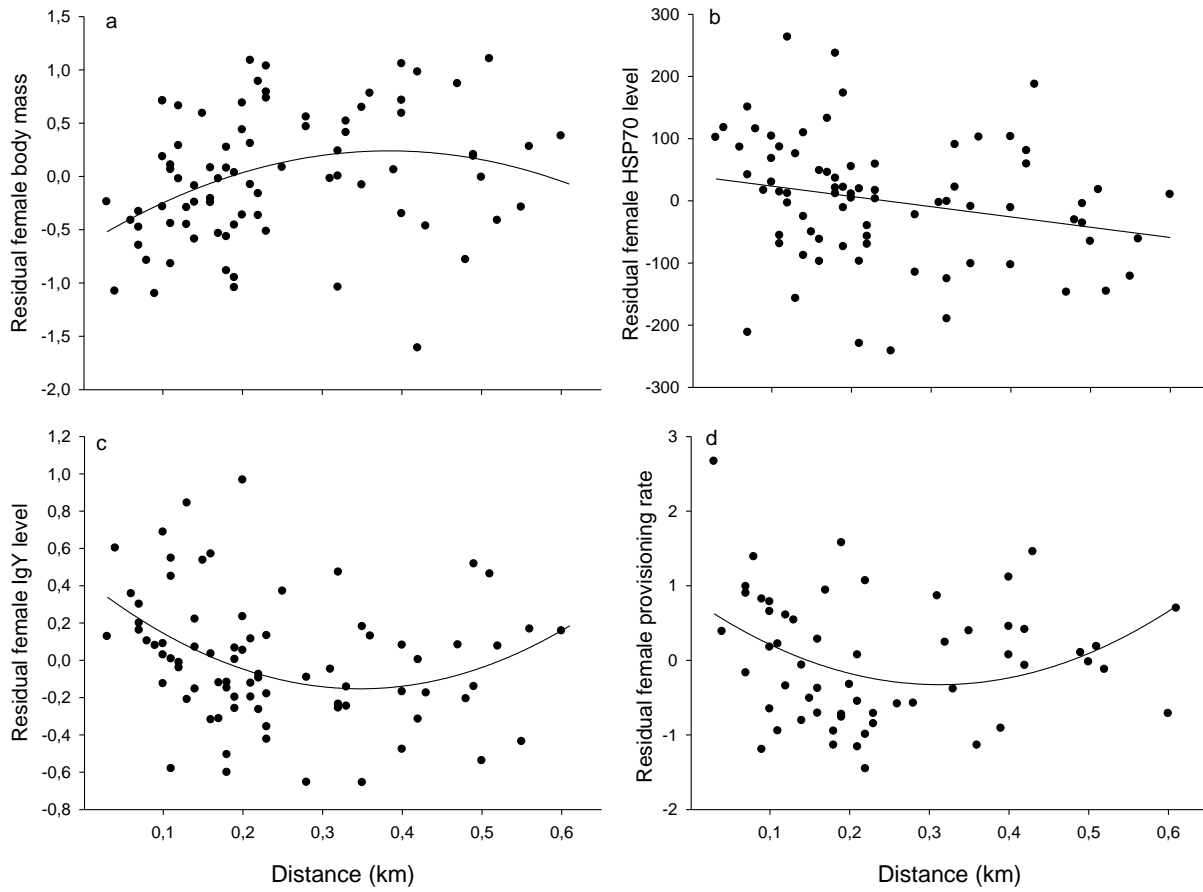| Variable	A: Nests from full range of distances			B: Nests closer than 300 m	
	30 m	Turning point	610 m	30 m	300 m
Body mass (g)	11.9	12.8 (at 391 m)	12.5	11.9	13.1
HSP 70	8091	-	6906	8527	6917
IgY	1.56	0.86 (at 353 m)	1.31	1.54	0.65
Provisioning (feeds/10min)	3.6	2.4 (at 322 m)	3.5	3.5	1.9

4

5

1   Figure Legend

2   Figure 1. Residual model values of the relationship between female pied flycatcher

3   physiological and behavioural measures taken during nestling provisioning with distance

4   of their nest from a sparrowhawk nest for a) body mass controlled for body size b) stress

5   protein (HSP70) levels, c) serum immunoglobulin IgY levels and d) female feeding rates

6   (feeds / chick / 10 mins).

7

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1   FIG. 1.