

# Balancing food and predator pressure induces chronic stress in songbirds

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The never-ending tension between finding food and avoiding predators may be the most universal natural stressor wild animals experience. The ‘chronic stress’ hypothesis predicts: (i) an animal’s stress profile will be a simultaneous function of food and predator pressures given the aforesaid tension; and (ii) these inseparable effects on physiology will produce inseparable effects on demography because of the resulting adverse health effects. This hypothesis was originally proposed to explain synergistic (inseparable) food and predator effects on demography in snowshoe hares (*Lepus americanus*). We conducted a 2 × 2, manipulative food addition plus natural predator reduction experiment on song sparrows (*Melospiza melodia*) that was, to our knowledge, the first to demonstrate comparable synergistic effects in a bird: added food and lower predator pressure in combination produced an increase in annual reproductive success almost double that expected from an additive model. Here we report the predicted simultaneous food and predator effects on measures of chronic stress in the context of the same experiment: birds at unfed, high predator pressure (HPP) sites had the highest stress levels; those at *either* unfed *or* HPP sites showed intermediate levels; and fed birds at low predator pressure sites had the lowest stress levels.

**Keywords:** chronic stress; food supplementation; predator pressure; synergistic effects; *Melospiza melodia*

## 1. INTRODUCTION

For sound logistical reasons population-scale experiments on terrestrial vertebrates have focused on one limiting factor at a time and most experiments on birds have focused on food effects (Newton 1998). Virtually all these experiments have been conducted in environments, or under circumstances, where predator pressure is low (Zanette *et al.* 2003). We conducted a bifactorial experiment to test whether birds responded equally to food addition in high predator pressure (HPP) and low predator pressure (LPP) environments. Our results showed that food and predators did not operate in an additive way, but instead had an interactive (or ‘synergistic’) effect on annual reproductive success (Zanette *et al.* 2003). The combined effect of added food and LPP produced an increase in annual reproductive success almost twice that expected from an additive model. To our knowledge, this is the first experimental study to show such synergistic effects in birds.

The first experimental study to demonstrate synergistic food and predator effects on the demography of any terrestrial vertebrate was published by Krebs *et al.* (1995). This study involved a bifactorial experiment on snowshoe hares. The combined effect of adding food and removing predators produced densities 1.9 times greater than that expected from an additive model. The hare results were completely unexpected and the ‘chronic stress’ hypothesis (Boonstra *et al.* 1998) was proposed to explain the mechanism responsible (Krebs *et al.* 2001). The ‘chronic

stress’ hypothesis predicts that: (i) an animal’s stress profile will be a simultaneous function of both food and predator pressures as a consequence of the never-ending tension between finding food and avoiding predators; and (ii) the inseparable effects of food and predators on physiology will result in inseparable effects on demography owing to the long-term adverse health effects of chronic stress. The snowshoe hare study ended before the ‘chronic stress’ hypothesis could be tested experimentally (Krebs *et al.* 2001).

The ‘chronic stress’ hypothesis is an extension of the ‘predator-sensitive foraging’ hypothesis (Hik 1995; Boonstra *et al.* 1998; Krebs *et al.* 2001). The results from the hare study were consistent with predictions from models (McNamara & Houston 1987; Abrams 1993) suggesting nonlinear (e.g. synergistic) changes in demography may be commonplace as a consequence of linear changes in the individual’s anti-predator and foraging behaviour. The difficulty in testing these models lies in the fact that behaviour is fleeting data collected at a given point in time that cannot be inferred to have lasting consequences. Sceptics can argue that the animal’s behaviour over the hour or two during which observations were conducted may be unrepresentative of what it is doing the rest of the time. The physiological stress effects proposed by the ‘chronic stress’ hypothesis provide the missing link between these short-term behavioural and longer-term demographic processes, the stress effects being the lasting ‘imprint’ of ‘predator-sensitive foraging’ (Boonstra *et al.* 1998). Results from literally hundreds of behavioural studies suggest that the constant tension between finding food and avoiding

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Table 1. Food and predator effects on measures of chronic stress.

(Up and down arrows signify the expected direction of change in each measure as predicted by the 'chronic stress' hypothesis, and that observed, in response to food shortage ( $\downarrow$  food) and/or increased predator pressure ( $\uparrow$  predators). Statistical results are main effects from two-way ANOVAs comparing fed versus unfed birds at HPP and LPP sites.)

| scale          | measure                 | change       |              | d.f. | $\downarrow$ food |          | $\uparrow$ predators |          |
|----------------|-------------------------|--------------|--------------|------|-------------------|----------|----------------------|----------|
|                |                         | expected     | observed     |      | <i>F</i>          | <i>p</i> | <i>F</i>             | <i>p</i> |
| hormonal       | maximum corticosterone  | $\uparrow$   | $\uparrow$   | 1,40 | 5.22              | 0.014    | 7.76                 | 0.004    |
|                | baseline corticosterone | $\uparrow$   | $\uparrow$   | 1,40 | 9.12              | 0.002    | 5.92                 | 0.010    |
| energetic      | FFAs                    | $\uparrow$   | $\uparrow$   | 1,35 | 4.58              | 0.020    | 5.08                 | 0.015    |
|                | glucose                 | $\uparrow$   | $\uparrow$   | 1,29 | 13.55             | 0.001    | 0.72                 | n.s.     |
| haematological | anaemia                 | $\uparrow$   | $\uparrow$   | 1,41 | 2.99              | 0.046    | 4.13                 | 0.025    |
|                | polychromasia           | $\uparrow$   | $\uparrow$   | 1,42 | 6.94              | 0.006    | 0.77                 | n.s.     |
| immunological  | basophils               | —            | $\uparrow$   | 1,42 | 0.28              | n.s.     | 6.74                 | 0.013    |
|                | H:L ratio               | $\uparrow$   | —            | 1,42 | 0.59              | n.s.     | 0.40                 | n.s.     |
| reproductive   | nestling brood size     | $\downarrow$ | $\downarrow$ | 1,65 | 5.35              | 0.012    | 0.22                 | n.s.     |
|                | nestling FA             | $\uparrow$   | $\uparrow$   | 1,43 | 0.14              | n.s.     | 5.59                 | 0.009    |

predators afflicts animals in virtually every vertebrate taxon (reviewed in Lima 1998). Inseparable food and predator effects on demography ought then to be the norm in both birds and mammals if the 'chronic stress' hypothesis is correct. Having demonstrated inseparable (synergistic) food and predator effects on the demography of song sparrows (Zanette *et al.* 2003) paralleling those shown in snowshoe hares (Krebs *et al.* 1995), we undertook to test whether each individual's stress profile was a simultaneous function of food and predators, as predicted by the 'chronic stress' hypothesis.

## 2. METHODS

### (a) *Experimental design*

Our study was conducted in the context of the same experiment described in Zanette *et al.* (2003). We monitored 91 song sparrow territories for the entire breeding season at 16 study sites near Victoria, British Columbia, Canada in 2002. Song sparrows in this area are resident and multi-brooded. Breeding begins in late March and ends in late July. Individuals can rear up to four broods of 1–4 young per year. We conducted a standard 2 (fed or unfed) by 2 (HPP or LPP) experiment. Added food, consisting of high fat and high protein (45%) pellets (Purina Mills Aquamax Grower 400) and millet was provided *ad libitum* throughout the breeding season (1 March onwards) to all of the territories at half of the sites. HPP sites (three fed plus three unfed) were located on the Vancouver Island (32 137 km<sup>2</sup>) 'mainland', while LPP sites (five fed plus five unfed) were less than 20 km distant, on several small (less than 200 ha) coastal islands. There were no significant differences between HPP and LPP sites in either nesting density (nearest neighbour distances: HPP = 52.7  $\pm$  4.7 m; LPP = 62.5  $\pm$  5.3 (mean  $\pm$  s.e.m.);  $t_{89} = 1.38$ ,  $p = 0.179$ ) or microsatellite heterozygosity (L. Zanette, unpublished data) and no significant difference in extra-pair paternity rates between fed birds at the HPP and LPP sites (L. Zanette, unpublished data). HPP sites supported a greater diversity and abundance of potential predators (Zanette *et al.* 2003) and song sparrows at HPP sites demonstrated significantly higher nest predation (68% versus 55%, HPP–LPP), higher brood parasitism rates (40% versus 9%), lower survival from fledging to independence (53% versus 82%) and lower adult breeding season survival (84% versus 92%) (Zanette *et al.* 2003; L. Zanette, unpublished data).

### (b) *Measures of chronic stress*

We tested for stress effects at five separate scales: hormonal, energetic, haematological, immunological and reproductive. We evaluated two measures at each scale. The authors of the 'chronic stress' hypothesis (Boonstra *et al.* 1998) predicted changes in all of these measures and in most cases they also predicted the expected direction of change (table 1). Changes in the same direction are predicted in response to food shortage ( $\downarrow$  food) or increased predator pressure ( $\uparrow$  predators), because *either* is potentially stressful. The critical prediction of the 'chronic stress' hypothesis is that *both* food and predators simultaneously affect stress levels.

The most direct measure of chronic stress, among those we evaluated, is the maximum concentration of the principal stress hormone, corticosterone, recorded in response to a standard stressor. The standard stressor used in most songbird studies, known as the 'capture stress protocol' (Wingfield *et al.* 1995), involves restraining the animal for a set period of time post-capture. Extensive prior research on song sparrows has demonstrated that corticosterone levels are maximal in blood collected 30 min post-capture (Wingfield *et al.* 1995). Baseline corticosterone, established by collecting blood less than 3 min post-capture (Scheuerlein *et al.* 2001), refers to concentrations in animals going about their daily routine. Baseline corticosterone may be biased if stressful events (unknown to the experimenter and unassociated with capture) occur immediately prior to capture. Maximum corticosterone is less likely to be biased by immediate events and is therefore a truer measure of chronic stress because levels are generally thought to be contingent on long-term enlargement of the adrenals (Boonstra *et al.* 1998).

The authors of the 'chronic stress' hypothesis predicted that chronically stressed animals should have a greater ability to mobilize energy for immediate muscle use at the expense of 'maintenance' or reproduction (Boonstra *et al.* 1998). In birds, free fatty acids (FFAs) power the flight muscles and glucose powers the leg muscles (Butler & Bishop 2000). Accordingly, both FFA and glucose levels should be higher in chronically stressed birds (table 1).

Anaemia (inverse of packed cell volume (PCV)) may be expected in response to chronic stress as a result of red blood cell (RBC) loss attributable to ulcers, high blood pressure or poor initial cell formation (Campbell 1988). Polychromasia is an index of the proportion of RBCs that are immature, reflecting RBC regeneration (Campbell 1988).

The authors of the 'chronic stress' hypothesis predicted changes in the composition of white blood cells (WBCs) reflecting changes in immune function in response to chronic stress (Boonstra *et al.*

1998). They did not predict which WBCs would predominate (table 1). In birds, a higher heterophil to lymphocyte (H:L) ratio is commonly used as an index of general stress based on poultry studies showing elevated H:L ratios in response to exogenous corticosterone (Carsia & Harvey 2000; see also McFarlane & Curtis 1989).

Chronic stress may affect reproductive rates by reducing either the quantity or quality of offspring produced. The authors of the 'chronic stress' hypothesis proposed using fluctuating asymmetry (FA) to judge offspring quality (Boonstra *et al.* 1998). FA is a measure of deviation from symmetry in bilateral organisms gauged by differences in paired appendages (e.g. arms or legs (Palmer 1994)). Greater stress is expected to result in more frequent developmental anomalies and greater FA (table 1).

### (c) Sampling

We captured and collected blood from fathers with 6 day old nestlings at 46 territories (10–13 per treatment). We conducted the capture stress protocol on fathers to avoid any potentially adverse effects on nestlings that might result from restraining the mother. Birds were captured using mist-nets. Up to 150 µl of blood was collected from the brachial vein less than 3 min from the time the animal hit the net. Blood from this first (baseline) bleed was used for all physiological (i.e. excluding reproductive) measures except maximal corticosterone concentration (table 1). The latter was measured in blood from a subsequent bleed conducted 30 min post-capture (Wingfield *et al.* 1995). Birds were held in cloth bags in the intervening period. All animals were bled at 10 min post-capture and eight were bled at 60 min post-capture to verify (Wingfield *et al.* 1995) that corticosterone levels increased from 10 to 30 min (paired  $t_{42} = -9.43$ ,  $p < 0.001$ ) and did not increase further after more than 30 min (paired  $t_7 = 1.79$ ,  $p = 0.117$ ). Glucose was measured and blood smears were prepared within 2 min of the first bleed. All remaining blood was stored on ice for transport to the laboratory. All samples were centrifuged, measured for PCV, and plasma was extracted and frozen at  $-20^{\circ}\text{C}$ , within 8 h. As reproductive measures (table 1) of potential stress effects at this stage of the breeding cycle we tallied brood size and measured the right and left tarsus lengths of all nestlings to compare levels of FA. Tarsus lengths were each measured twice, to the nearest 0.01 mm, and analyses were restricted to measurements made by a single observer (Palmer 1994).

### (d) Laboratory analyses

Radioimmunoassays of corticosterone concentrations were conducted using 5–20 µl of plasma following extraction in dichloromethane (Wingfield *et al.* 1992). Plasma FFA concentrations were determined using the NEFA C test kit (Wako Chemicals, Neuss, Germany) (Johnson & Peters 1993). Glucose was measured using the ONE TOUCH Ultra (LifeScan Canada Ltd, Vancouver, Canada). Smears were stained using Wright's stain and polychromasia and WBC differentials were evaluated by trained technicians at the Animal Health Laboratory, Ontario Veterinary College, University of Guelph (Guelph, Canada). Basophils constituted  $11.9 \pm 1.0\%$  (mean  $\pm$  s.e.) of all WBCs. While basophil counts are typically lower in poultry, higher counts are found in other species (King & McLelland 1984) and may be the norm in New World sparrows (Ruiz *et al.* 2002). Eosinophils and monocytes each constituted less than 5% of all WBCs ( $3.7 \pm 0.6\%$  and  $1.3 \pm 0.3\%$ , respectively). Eosinophils and monocytes are difficult to distinguish from heterophils and lymphocytes, respectively (Campbell 1988). Separate analyses of the H:L ratio were conducted either pooling ((het. + eos.):

(lym. + mono.); table 1) or discriminating (het.:lym. only) difficult to distinguish WBCs. There was no effect of either pooling or discriminating on the outcome of analyses.

### (e) Data analyses

We conducted two-way ANOVAs of all the measures in table 1 comparing fed versus unfed birds at HPP and LPP sites. Only main effects are reported because only one of the interaction terms (figure 1f) approached significance ( $p > 0.10$  in all other cases). Results in table 1 are one-tailed where *a priori* predictions exist. Prior to analysis, all data except  $|R-L|$  tarsus length were Box-Cox transformed (Krebs 1999) and tested for normality and homogeneity of variances. Relevant regressions (stepwise, where multiple independent variables) were conducted of time (seconds) from capture to bleed, time (minutes) of day, date and/or brood size, versus each measure. There was no significant correlation between baseline corticosterone and time from capture to the first bleed ( $r^2 = 0.05$ ,  $t_{43} = 1.46$ ,  $p = 0.151$ ), nor were any other regressions significant except in the case of FA. Degrees of freedom for the physiological measures in table 1 vary because of occasional sample losses. Analyses of brood size and FA include all available data from the larger demographic study. Where there was more than one day 6 brood per territory the average day 6 brood size for the territory was used. Meaningful FA (Palmer 1994; Palmer & Strobeck 2003) was indicated by a significant 'sides  $\times$  individuals' interaction ( $F_{48,98} = 3.92$ ,  $p < 0.001$ ; repeatability [ME5]  $r_A = 0.056$ ), using data from one randomly-selected nestling per brood. Average FA per brood showed a negative correlation ( $r^2 = 0.28$ ,  $t_{46} = -4.27$ ,  $p < 0.001$ ) with date and a positive correlation ( $r^2 = 0.45$ ,  $t_{46} = 6.12$ ,  $p < 0.001$ ) with brood size. Consequently, date and brood size were included as covariates when analysing effects on FA.

## 3. RESULTS

Both food and predators significantly affected the individual's stress hormone profile, as predicted by the 'chronic stress' hypothesis. Both maximum (figure 1a) and baseline (figure 1b) corticosterone levels varied significantly with both food and predators (table 1). The direction of change was also as predicted (table 1), being greater at unfed than at fed sites and greater at HPP sites than at LPP sites (figure 1a,b).

Both food and predators also significantly affected the individual's energetic profile. FFA levels varied significantly with both food and predators (table 1), showing the same pattern as corticosterone levels (figure 1c). Food alone affected glucose levels (table 1; fed =  $361.1 \pm 5.6 \text{ mg dl}^{-1}$ ; unfed =  $393.8 \pm 6.4$ ).

Both food and predators significantly affected the individual's haematological profile, in accordance with the 'chronic stress' hypothesis. Anaemia varied significantly with both food and predators (table 1), showing the same pattern as corticosterone and FFA levels (figure 1d). Food alone affected polychromasia (table 1; fed =  $5.5 \pm 0.5\%$ ; unfed =  $7.5 \pm 0.6$ ).

Predator pressure alone affected our immunological measures. Basophils made up a significantly greater proportion (table 1) of WBCs at HPP sites ( $14.5 \pm 1.5\%$ ) than at LPP sites ( $9.4 \pm 1.3\%$ ). Neither food nor predators affected the H:L ratio (table 1). There was no significant correlation between the H:L ratio and either maximum ( $r^2 < 0.01$ ,  $t_{42} = 0.40$ ,  $p = 0.688$ ) or baseline ( $r^2 = 0.03$ ,  $t_{43} = 1.16$ ,  $p < 0.254$ ) corticosterone concentrations.

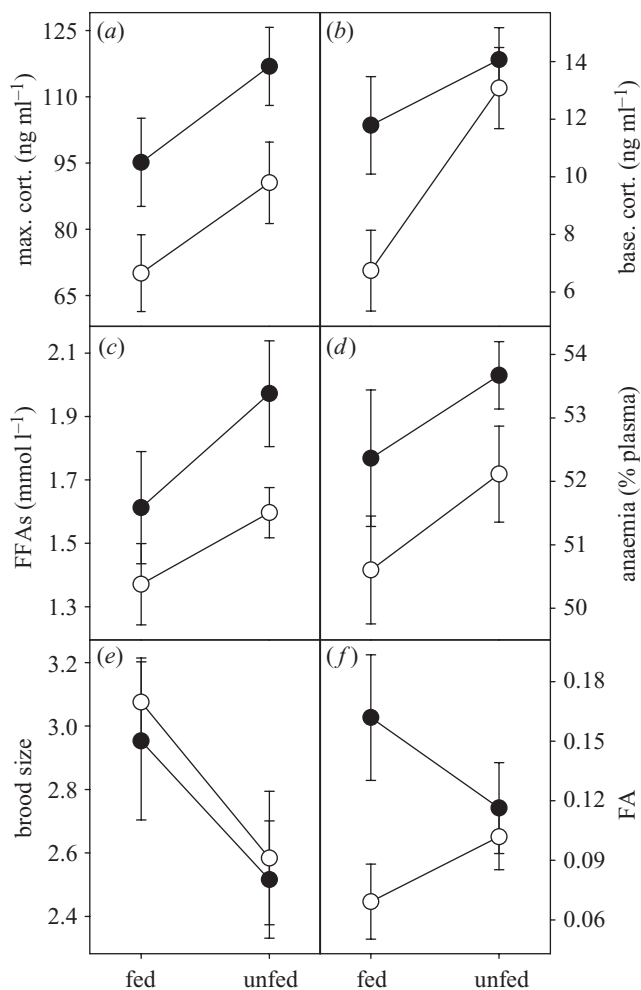


Figure 1. Measures of chronic stress in song sparrows on day 6 of the nestling period at fed and unfed sites subject to high (closed circles) and low (open circles) predator pressure. Values are means  $\pm$  s.e.m. (a) Maximum plasma corticosterone concentration; (b) baseline plasma corticosterone concentration; (c) plasma FFA concentration; (d) anaemia (percentage plasma in hematocrit); (e) average nestling brood size; and (f) average FA in nestling tarsus lengths, per brood.

Food and predators affected the quantity and quality, respectively, of day 6 nestlings. Broods at unfed sites were significantly smaller than those at fed sites (figure 1e; table 1) and average FA in nestling tarsus lengths per brood was significantly greater at HPP sites than at LPP sites (figure 1f; table 1). The direction of change in both cases was as predicted by the 'chronic stress' hypothesis (table 1).

#### 4. DISCUSSION

Chronic stress in response to *both* food and predators was evident at the hormonal, energetic, hematological and reproductive scales (table 1). Song sparrows at the unfed, HPP sites showed the highest stress levels (figure 1a–d), birds subject to *either* the unfed *or* HPP treatments showed intermediate stress levels, and fed birds at the LPP sites showed the lowest stress levels (figure 1a–d). Thus, the stress profile of parental male song sparrows on day 6 of the nestling period clearly appears to be a simultaneous function of food and predators, as predicted by the 'chronic stress' hypothesis.

We are not aware of any prior field study showing effects on corticosterone in response to food addition in songbirds despite the numerous laboratory studies on corticosterone effects on songbird foraging (Wingfield & Silverin 2002). Similarly, while several songbird studies have shown acute activation of the stress axis in response to the experimental presentation of a predator (Silverin 1998; Canoine *et al.* 2002; Cockrem & Silverin 2002), only one has tested whether predators can induce chronic activation of the stress axis. Scheuerlein *et al.* (2001) reported that tropical stonechats (*Saxicola torquata axillaris*) with predatory fiscal shrikes (*Lanius collaris*) in their territories had significantly higher baseline corticosterone than those without shrikes. In contrast to the strong effect of predators on maximum corticosterone that we observed (figure 1a), shrikes did not affect maximum corticosterone in the stonechats. The simplest explanation of this apparent difference is that our HPP versus LPP treatment involved a suite of predators rather than just one.

While food effects on the energetic profile of songbirds have been reported previously (Jenni & Jenni-Eiermann 1996; Totzke *et al.* 1998) we are not aware of any other study examining predator effects. The finding that food affected both FFAs (figure 1c) and glucose (table 1) is consistent with the fact that song sparrow foraging involves both flying and hopping, entailing the use of FFAs and glucose respectively. The finding that predator pressure affected FFAs alone (table 1) is consistent with predator evasion relying primarily on FFA-dependent flight muscles rather than glucose-dependent leg muscles (Butler & Bishop 2000).

As with energetics, food effects on the haematological profile of songbirds are well documented (Hoi-Leitner *et al.* 2001; Cucco *et al.* 2002) while predator effects have been neglected. Because both polychromasia and anaemia were greater at unfed sites (table 1) RBC regeneration (polychromasia) may have been compensating for RBC loss (anaemia), whereas the effect of predator pressure on anaemia alone (table 1) suggests RBC regeneration was not compensating for RBC loss at HPP sites and the haematological profile of birds at these sites was actually worse than that indicated by anaemia alone (figure 1d).

While there has been much research on whether the immune response affects vulnerability to predators in songbirds (Moller & Erritzoe 2000), the significant effect of predator pressure on basophils reported in table 1 is, to our knowledge, the first demonstration of predator effects on the immune response. Like most avian WBCs the function of basophils is largely unknown (Campbell 1988). At present, therefore, there is no way to judge the importance of this result. Despite its common usage, the adequacy of the H:L ratio as an indicator of general stress has rarely been evaluated in wild birds (Ots *et al.* 1998). Given the lack of correlation between the H:L ratio and corticosterone in song sparrows we suggest caution in the interpretation of the H:L ratio as an indicator of general stress in species for which this has not been corroborated.

Increased brood size in response to experimental food addition (figure 1e) has been shown before in song sparrows (Arcese & Smith 1988). While predator pressure alone significantly affected FA (table 1) there was an obvious trend ( $p = 0.079$ ) towards an interactive effect of food and predators (figure 1f). FA increases with brood size (see § 2e) suggesting the strain of producing more offspring

results in poorer quality offspring. Because food addition increases brood size it may also increase FA. The results in figure 1f are, however, corrected for brood size. Food addition also significantly increases the number of broods per season (Arcese & Smith 1988; L. Zanette, unpublished data). Both food and predators may affect FA if the strain of producing more broods in response to food addition is exacerbated at HPP and attenuated at LPP sites (figure 1f).

There is a growing literature on the effects of early nutritional stress on neuronal development and resultant adult learning disabilities in birds (Nowicki *et al.* 1998; Kitaysky *et al.* 2003). Higher FA at HPP sites (table 1) may result from predator effects on parental foraging. Mothers spend significantly more time guarding the nest on day 6 of the nestling period at HPP sites (L. Zanette, unpublished data), which presumably limits their ability to find food for themselves and their young. If predator-induced nutritional stress affects FA this could also affect neuronal development and adult learning ability. Song learning significantly affects pairing success in song sparrows (Nowicki *et al.* 2002). Thus, both food- (Nowicki *et al.* 1998) and predator-induced stress on parents may affect the pairing success of their sons.

Our measures at different scales reflect different points on the 'stress axis' (see Boonstra *et al.* 1998, fig. 1; Romero 2004, fig. 1). Elevated corticosterone should have cascading effects on energetics, haematology, immunology and reproduction. Eight of our 10 measures are repeated measures of the same individual. If head length, arm length and leg length were consistently greater in group A than B, we would conclude individuals in group A were larger, even if the differences in each measure were slight. Correcting for multiple comparisons would clearly be inappropriate. Similarly, evidence of chronic stress is clearly more obvious the more points on the 'stress axis' are affected. Measures at different scales represent multiple tests of the same hypothesis. In this case, a Fisher's combined probabilities test (Sokal & Rohlf 1995) is the most correct statistical procedure to follow. Such a test is, however, manifestly redundant, given the dramatically significant results at so many different scales (table 1).

We based our selection of HPP and LPP sites on known differences in predation rates on nearby island and mainland song sparrow populations (Arcese *et al.* 1992; Smith *et al.* 1996; Rogers *et al.* 1997) and the general observation that predator pressure is lower on islands (Palkovacs 2003). Given our *a priori* selection of sites likely to differ in predator pressure, the fact that those sites do differ in predator pressure (see § 2a) and the absence of obvious alternatives (see § 2a), we think it entirely reasonable to ascribe the observed differences in stress levels (open versus closed circles in figure 1) to differences in predator pressure. Similarly, we can reject alternative explanations of our results as being attributable to among treatment differences in the point in the reproductive cycle sampled or differences associated with circadian or seasonal fluctuations in hormone levels because we controlled for the point in the reproductive cycle sampled in our experimental design by sampling all males on the same day (day 6) during the nestling period (see § 2c), and potential biases associated with time of day or date were found to be non-significant in our statistical analyses (see § 2e).

A simple example illustrates how nonlinear changes in demography result from linear changes in behaviour. In theory, doubling time spent vigilant will halve the individual's time spent foraging, resulting in a decreased probability of death owing to predation and increased probability of death owing to starvation (McNamara & Houston 1987; Abrams 1993). Total mortality will remain unchanged only in the peculiar event that predation and starvation are linearly proportional. If a unit increase in time spent vigilant decreases predation faster than the unit decrease in time spent foraging increases starvation, total mortality will decrease (McNamara & Houston 1987; Abrams 1993). In this case demography (total mortality) is not a simple linear function of linear changes in behaviour. Similarly, there is no reason to expect that nonlinear (e.g. synergistic) changes in demography can only result from nonlinear (interactive) physiological effects. Indeed, Romero (2004) has recently argued that linear physiological responses such as we observed may result from nonlinear physiological processes. The focus of the 'chronic stress' hypothesis, like the 'predator-sensitive foraging' hypothesis, is the inseparable link between food and predators at the individual scale rather than the linearity or nonlinearity of the resulting physiological or behavioural phenomena. Synergistic food and predator effects on demography demonstrate an inseparable link at the population scale. Showing inseparable food and predator links at both the individual and population scales is the critical step in testing the 'chronic stress' hypothesis.

At the scale of the individual, chronic stress is normally associated with pathology. In our study, euthanizing individuals to look for tissue damage would mean sacrificing our ability to simultaneously test for demographic effects. Larger sample sizes than ours would be necessary to permit subsampling for pathology. While chronic stress may induce pathology it is not necessarily maladaptive. Rather, the organism may be 'making the best of a bad job'. Elevated corticosteroid levels are very adaptive when behaviour must be redirected from resting to running, for example, when face to face with a predator. 'Allostasis', defined as maintaining stability through change, has been proposed as an alternative to 'stress', to avoid the connotations of maladaptation associated with the latter term (McEwen & Wingfield 2003). 'Allostatic overload' in turn refers to situations where the organism's ability to maintain internal stability is exceeded. Allostatic overload may result from either acute situations where energy demand exceeds supply (type 1), or chronic situations where no clear alternative behavioural response can alleviate the threat (type 2). The pathological consequences of the latter can be seen in the illnesses that often afflict animals in captivity. We suggest wild animals face an analogous challenge in being unable to escape the necessity of finding food in an environment where the threat of death is always imminent. Physiological and behavioural compromises must be made. The truly maladaptive options are either not to find food or to ignore the predators.

In this study we have, in a sense, been working backwards. After first demonstrating inseparable (synergistic) food and predator effects on demography (Zanette *et al.* 2003), we have now verified the presence of the individual-level mechanism predicted by the 'chronic stress' hypothesis to be responsible. The next step is to determine which

demographic parameters are most affected. What we find most compelling about our data is how well the results concerning song sparrows correspond with predictions originally derived from work on snowshoe hares (table 1). Given the numerous differences between these species, this speaks volumes for the probable generality of these phenomena. The underlying behavioural link, being the never-ending tension between finding food and avoiding predators, is acknowledged as being nearly universal (Lima 1998). There is no reason to expect the stress axis differs dramatically among organisms (Sapolsky *et al.* 2000). Thus, there is every reason to expect that the 'chronic stress' hypothesis should apply to the majority of vertebrates. Because the individual-level mechanism is very likely nearly universal, the resulting demographic effects should be as well. Consequently, we suggest future demographic studies begin by assuming food and predator effects are inseparable. Indeed, this may be critical for species protection. Conservation efforts aimed at either food or predators are often disappointing (Zanette 2000; Zanette *et al.* 2000, 2003). Simultaneously targeting both may not only be the key, but could also provide disproportionate benefits per dollar spent given the more than additive demographic responses shown in both song sparrows and snowshoe hares.

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