Predatory Bird Monitoring Scheme
http://pbms.ceh.ac.uk/

# The potential for the use of population health indices in the Predatory Bird Monitoring Scheme: a Predatory Bird Monitoring Scheme (PBMS) report

**L.A. Walker, E.D. Potter, L.H.V. Franklinos#, R.J. Strong$, J.S. Chaplow, M.G. Pereira & R.F. Shore[1]**

**Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK; #Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, UK; $ University of Lancaster, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4YQ, UK.**

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[1]Corresponding author: RF Shore, Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. E-mail: rfs@ceh.ac.uk. Website: http://www.ceh.ac.uk/

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# Contents

# 1. Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability activities for contaminant monitoring and surveillance work on avian predators. The PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Each bird that is submitted to the scheme is given a post-mortem examination during which approximately 60 macroscopic observations and measurements are made. The information gathered during this examination could potentially be used to monitor health status of the birds at the time of their death or at a particular stage of their development. In the current study, we focused on examining potential health indicators for the sparrowhawk, *Accipiter nisus*, as a candidate species partly because we have a long track record of collecting carcasses and eggs of this species, and so hold a substantial associated post-mortem (PM) observations and egg morphometric data sets. This species is sexually dimorphic, another reason for using it as a candidate species as it allowed us to investigate if the various health indices would need to be (and could be) defined separately by age class and sex.

We were able to establish baseline "norms" in the form of Shewhart charts for indicators that could be broadly categorised as indicators of change in: (i) population demography because of altered recruitment, survival and mortality (measures were sex ratio, proportion of first-year birds, proportion deaths from starvation or disease, eggshell index); (ii) nutritional status (measures were body weight, fat score, condition index) and (iii) physiological stress (fluctuating asymmetry). The measurements necessary to calculate these indices are routinely captured by the PBMS through direct input into an Oracle database at the time of PM examination. We also explored the potential for annual monitoring of feather corticosterone as a simple effects biomarker for environmental stress, including environmental contaminants, but further work and resource would be needed to incorporate any such measure into annual health surveillance monitoring.

We outline how the health indices described here could be reported in real-time and extended to other species to provide surveillance across different trophic strategists, and ecosystems. This report is intended to prompt debate about the type of population health indices that may be of use in assessing environmental health. It is not intended to be definitive in terms of which should be used.

# 2. Introduction

## 2.1. Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and, in particular, to vertebrate wildlife. Our monitoring provides scientific evidence of how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory and policy decisions about sustainable use of chemicals. In addition, the outcomes from our monitoring are used to assess whether effects are likely to occur in wildlife, whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently the PBMS has two key general objectives:

(i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value

(ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the PBMS website.

## 2.2. Health indices and aims of the current study

Each bird that is received by the PBMS undergoes a post-mortem examination (PM). In addition, tissue samples are collected both for chemical analyses in current projects and/or for retention in our long-term tissue archive (Walker *et al.*, 2010, Walker *et al.*, 2014); archived samples are often used in retrospective ecotoxicological and ecological studies.

Approximately 60 macroscopic observation and measurements are made during the PM. To date, the main measurements that have been used have been those for species, age, sex and nutritional status as we have examined how these factors affect exposure to and accumulation of contaminants (for instance Crosse *et al.*, 2013, Wienburg and Shore, 2004). However, the wider information gathered during the PM can potentially be used to assess the health status of birds at the time of their death. Shewhart (control) charts for various parameters can be generated from previously collected PM data and used to assess whether metrics collected in future years are within or without the normal range, or are changing systematically over time. Such evaluation, conducted for a range of PM metrics, could provide an overall indicator of the general health status of populations for any one year and over longer time periods.

To identify whether different PM measurements are likely to be suitable for monitoring wider health status, a number of questions need to be answered, namely:

1. Is it possible to establish a baseline “norm” for various PM measurements?
2. Can all birds be monitored together or does demographic group (age and sex of the bird) have an influence on the values for any health index?
3. Which demographic groups need to be monitored separately?
4. Is it possible to define “trigger values” for various health indices, deviation from which would indicate significant within year deviation from the norm?
5. Are the indices likely to be biologically meaningful?

In this study, we addressed the above questions for the following indices in sparrowhawks (*Accipiter nisus*):

- Number submitted, sex ratio, age ratio and bodyweight of birds received by the PBMS
- the proportion of birds that have died of starvation and disease or were in a starved state
- the level of fluctuating asymmetry in morphological features
- eggshell indices

We chose the sparrowhawk as a candidate species partly because we have a long track record of collecting carcasses and eggs of this species, and so hold a substantial associated PM and egg morphometric data sets. This species is sexually dimorphic, another reason for using it in this project as it allowed us to address questions with regard potential variation in health indices between males and females and first year and adult birds. In addition to carcass and egg morphometric measurements, we also examined the potential for using feather corticosterone hormone levels as a potential health indicator in a range of species received by the PBMS.

This study was designed to be a preliminary assessment of the potential to use different health indices to monitor the overall health status of predatory bird populations. The purpose was to trial this using measurements made on sparrowhawks which, if considered feasible, could be applied to other species. The intention is to review the outputs from this report and seek the views of other potential users and stakeholders as to the value of the metrics outlined. The metrics may then be applied to other species and collated to provide a more holistic assessment of environmental health, as indicated by predatory birds.

# 3. Results and Discussion

## 3.1. Number of birds submitted to the PBMS

The PBMS receives between 300 and 400 birds a year with, in recent years, approximately 90% comprised of six species - barn owl (*Tyto alba*), sparrowhawk, tawny owl (*Strix aluco*), common buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*) and red kites (*Milvus milvus*).

We have records of the number of sparrowhawks received by the PBMS between 1963 and 2015 (Fig. 1). Numbers increased between 1963 and the early 1990s and then subsequently declined. It has previously been suggested that variation over time in the numbers of sparrowhawks submitted to the PBMS reflects change in population numbers (Newton *et al.*, 1999). The variation in carcass numbers received indeed seem similar to those published by the British Trust for Ornithology (BTO) based on their common bird census (CBC) and breeding bird survey (BBS). The BTO data for the period 1974 to 2014 indicates a peak in sparrowhawk population in England in the early 1990s through to the early 2000s followed possibly by a more recent decline (Fig. 2).

To investigate the correspondence between numbers of carcasses received and the BTO population estimates, we assigned rank scores to the annual values for both measures over the period 1974 and 2014 and compared the correspondence of the ranks (Fig. 3). There was a significant positive relationship between the rank scores ($r^2 = 0.24$, $F_{(1,37)} = 11.6$, $P=0.002$ (Figure 3, top graph), suggesting that falls in the numbers of sparrowhawk carcasses submitted to the PBMS may be indicative of reductions in population numbers. However, we then repeated the analysis but split the data into two time periods based on the trends in the BTO data, first for the period 1974-1994 over which sparrowhawk numbers rose steadily, and second the period post-1994 during which numbers had stabilized or fell somewhat (Fig. 2). This demonstrated that while the relationship between the ranked carcass and population number scores was highly significant in the period 1974-94, ($r^2 = 0.85$, $F_{(1,19)} = 107.9$, $P<0.0001$; Fig. 3 middle graph) as found by Newton *et al.* (1999), there was no such relationship post-1994 ($r^2 = 0.00$, $F_{(1,16)} = 0.007$, $P>0.05$; Fig. 3 bottom graph).

These analyses suggest that when sparrowhawks were relatively scarce, annual variations in number of carcasses submitted to the PBMS were largely associated with variation in population numbers in England. However, as sparrowhawks have become more abundant, there appears to be no obvious association between population indices and numbers of carcasses submitted to the PBMS. Presumably other factors, such as annual variation in collector effort and relative variation in the numbers of carcasses submitted from the three GB countries (in which population trends may differ), may be more important.

Given these analyses, it is not possible to define a metric in terms of sparrowhawk carcass submissions that would indicate a "norm", for which annual values above or below would indicate a meaningful change in population status. Variation in carcass submissions is likely to be increasingly affected by collector effort in the future as the PBMS enters new collaborations (for example between the PBMS and other carcass collection schemes in

Scotland and elsewhere). Therefore, simple carcass numbers are unlikely in their own right to be a good candidate for a health index.

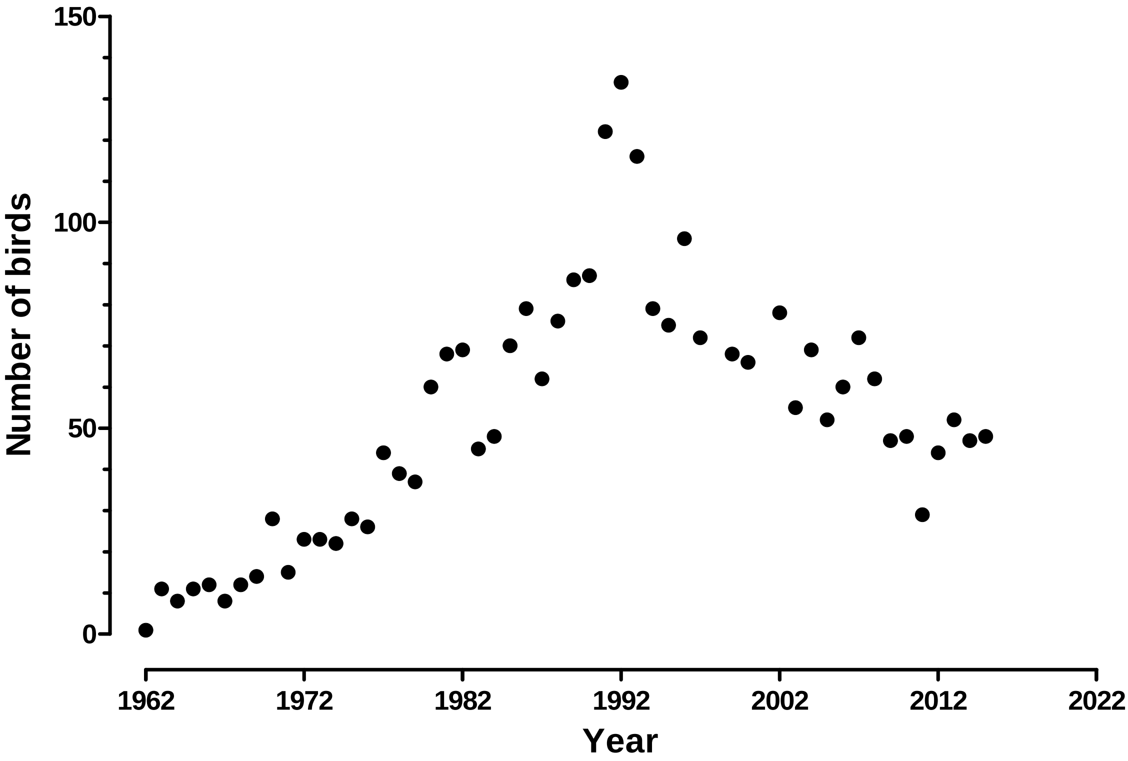


**Figure 1.** The number of sparrowhawks received by the PBMS between 1963 and 2015.

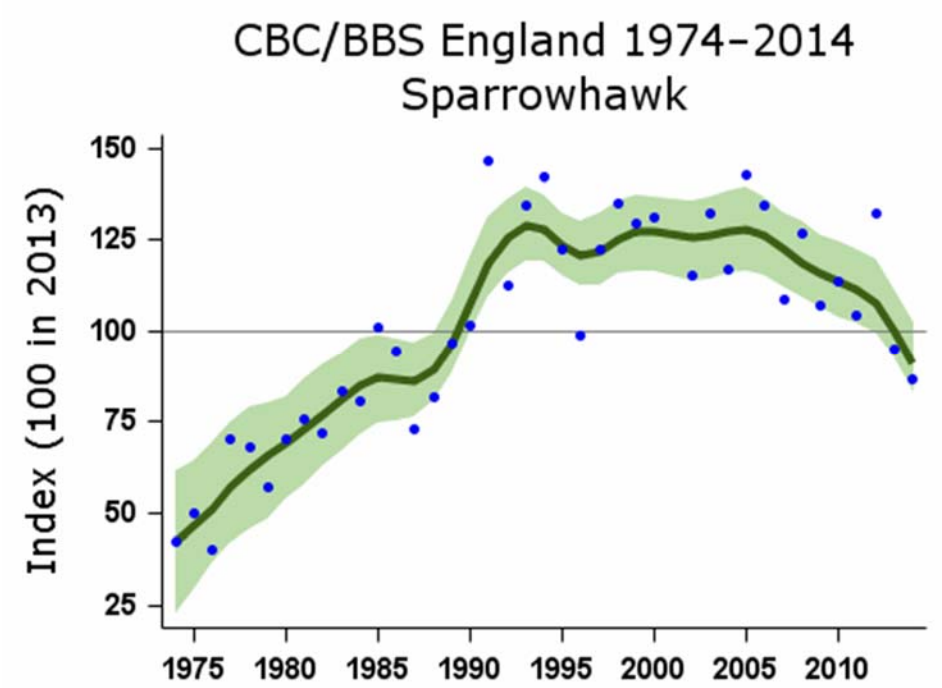


**Figure 2.** BTO population index for sparrowhawks in England based on the common bird

census and breeding birds survey (Taken from British Trust for Ornithology, 2016).

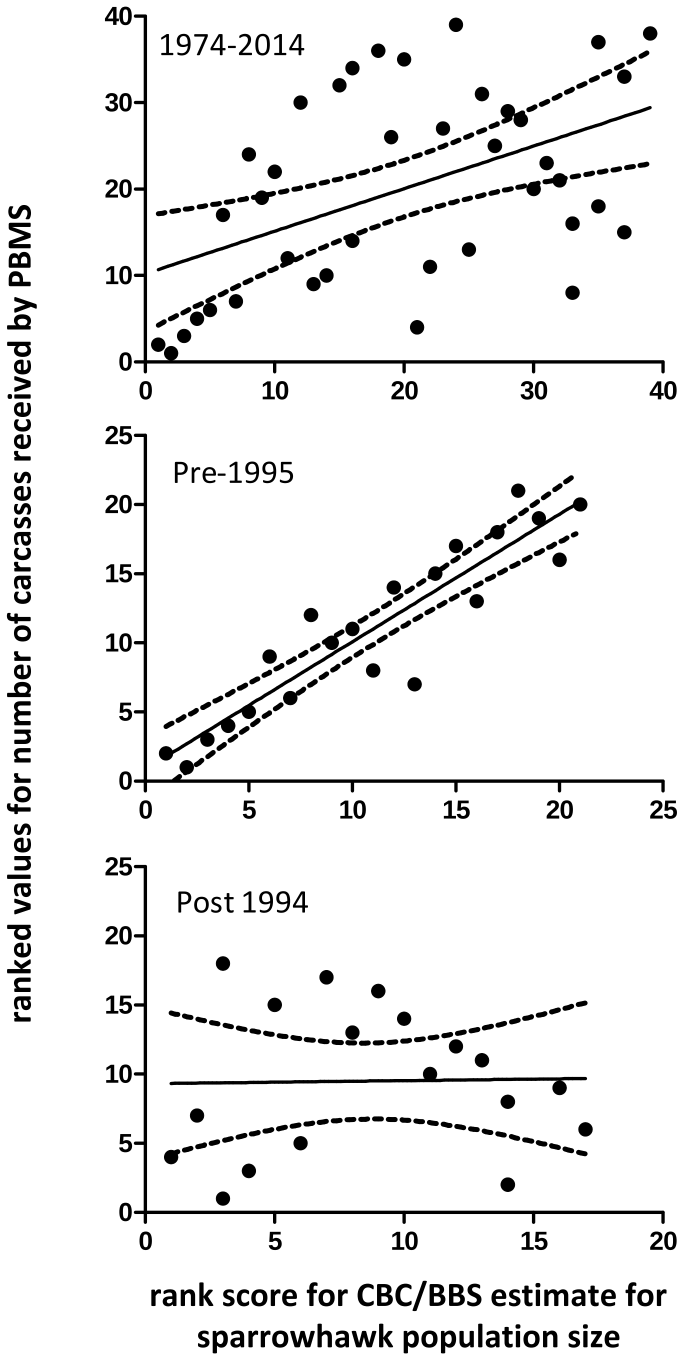


**Figure 3.** Rank of annual sparrowhawk numbers received by the PBMS plotted against rank CBC/BBS index score for sparrowhawks in England for the periods 1974-2014, (top graph), pre-1995 (middle graph) and post-1994 (lower graph).

## 3.2. Sex ratio

The sex of each bird submitted to the PBMS is determined during the necropsy and is based on body size, weight, plumage and positive identification of the gonads. Using our long-term data, we calculated the proportion of sparrowhawks that were female for all years in which the number of carcasses for which sex was determined was ≥ 5. Over the period 1966 to 2014, more female than male sparrowhawk carcasses were received by the PBMS in most years and the difference was statistically significant (Fisher's exact test, P=0.011). This preponderance of females contrasts with the reported sex ratio in fledglings between 1969 and 1976 (Newton and Marquiss, 1979) and in fledged birds in a study carried out in Northamptonshire between 1980 and 1989 (Wyllie and Newton, 1991); both studies reported a close to unity sex ratio, although Newton et al. (1986) found a preponderance of females in field studies in southern Scotland. However, the higher proportion of females in PBMS samples was consistent (no significant change over time: Pearson correlation coefficient r=0.01, P=0.945) and suggests that the sex ratio of birds received by the PBMS is not a true indicator of the sex ratio in the population. This may be because females hunt more on farmland and open habitats than males (Marquiss and Newton, 1982) and so are more likely to be found (Newton *et al.*, 1999). It is also possible that larger females are more readily noticed by collectors.

Even though the sex ratio of sparrowhawks received by the PBMS may not exactly equate to that in the wider population, the consistency in the proportion in the PBMS sample suggest that it still may be useful as an indicator of change in population structure over time. The average proportion of females was not normally distributed when data for all years was considered and so we used averages based on medians and prediction intervals based on the 1% and 99% percentiles; these are relatively close to 95% prediction intervals in datasets that are normally distributed. The annual median proportion for females was 0.551 and associated 99$^{th}$ percentiles were 0.28 and 0.81 (Fig. 4). Thus, future years in which the proportion of female sparrowhawk carcasses submitted is <0.28 or >0.81 could be considered unusual years and may be indicative of other changes to the status of birds.

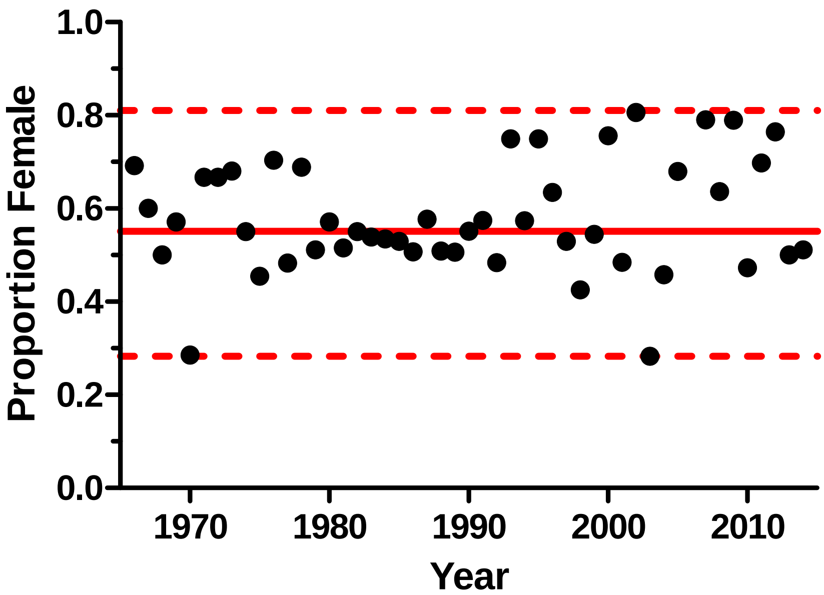


**Figure 4.** The proportion of birds received by the PBMS that were female. Red solid and dashed line indicates median and 99$^{th}$ percentiles, respectively.

## 3.3. Age ratio

The number of sparrowhawks submitted to the PBMS between 1968 and 2015 ranged from 10 to 132. At PM, birds are classified as either first year birds or adults where first years are defined as birds that hatched in the current or previous year. The increase over time in the proportion of first-year birds (Figure 5, top graph) was analysed by linear regression (with sex as a factor in the model) and was significant ($F_{1,93}$ = 19.00, P<0.001); there was no effect of sex ($F_{1,935}$ =1.55, P=0.21). Although the increase in the proportion of first-year birds appeared to level off in later years, comparison of linear vs quadratic models using Akaike's Information Criteria (AIC) indicated a linear model was the better fit.

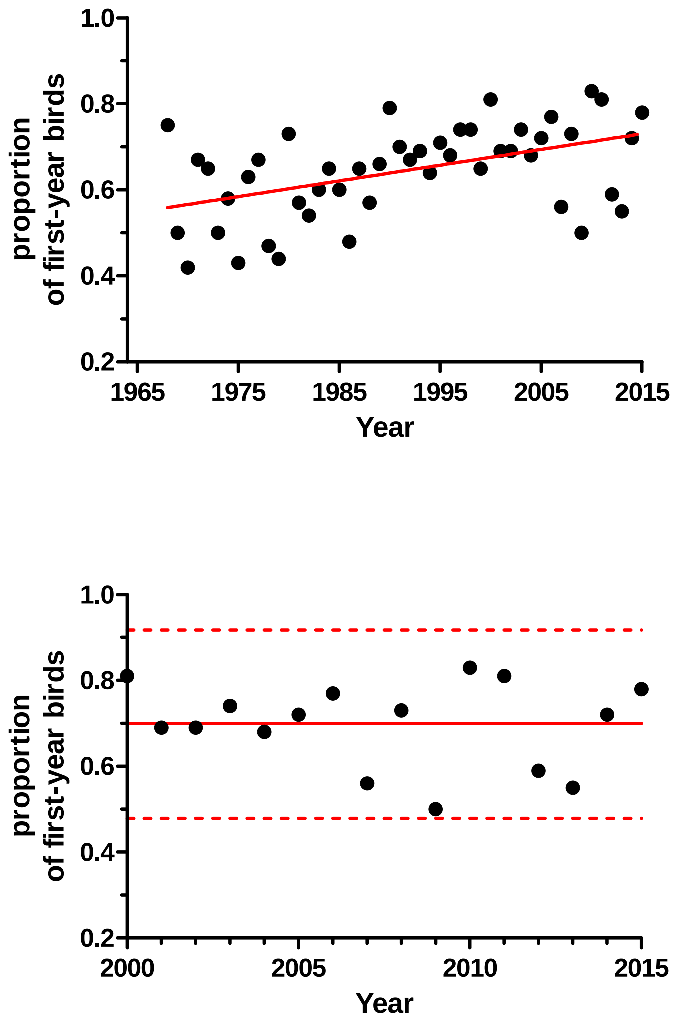


**Figure 5.** Proportion of sparrowhawks received annually that were first-year birds. Top graph: proportion of sparrowhawks received annually between 1968 and 2015 that were first-year birds. Linear regression: Proportion first-year birds = 0.0.84 +0.0039*(Year-1967), $R^2$=0.235. Bottom graph: proportion of sparrowhawks received annually between 2000 and 2015 that were first-year birds. Solid line indicates mean value and dotted lines are 95% prediction intervals.

The increase in the proportion of first year (juvenile) sparrowhawks in the samples of carcasses received by the PBMS has been attributed to a reduction in DDE contamination, an associated reduction in eggshell breakage, and a consequent increase in breeding success (Newton *et al.*, 1999). Although in our analysis, a linear increase over time was the regression model that gave the best fit to the data, such an increase clearly cannot be sustained indefinitely (as eventually, there would be no adults in the sample). If the increase over time is indeed due to recovery from DDE-mediated eggshell thinning, adult:first-year bird ratios might be expected to revert to a "norm" once eggshell thickness recovered to levels that were typical of those in pre-DDT years. Analysis of eggshell indices (see Section 3.9) indicated that sparrowhawk eggshell thickness was consistently within pre-DDE levels from the year 2000 onwards. This might account for the apparent leveling off in the proportion of first-year birds in the PBMS sample at around this time (Fig. 5 top graph). We therefore re-analysed the proportion of first-year birds in the annual PBMS collections but only for years from 2000 onwards (Fig 5, bottom graph). This indicated that there was no significant change over time for this period, and so it was possible to calculate the mean % of first-year birds per year and associated 95% prediction intervals (Figure 5, bottom chart). This value, and its prediction intervals, can be used to identify when the proportion of first-year birds in the population is lower than would be expected by chance either in a single year or as a trend over time. Such a finding might be indicative of poor juvenile recruitment into the population and it is proposed that this metric could be a useful indicator of the general "health status" of the population.

## 3.4. Body weight

The body weights of sparrowhawks received by the PBMS between 1977 and 2015 were first analysed to test if there were temporal trends and then to define prediction limits for average body weights for this species. Data for age/sex groups were summarised separately and data were only used for years in which the number of birds in a sex/age category was >5; in all cases the minimum number of years included was more than 25. Within year body weight data were often not normally distributed overall and so annual averages were based on median values for each year.

Spearman rank correlation analysis indicated there was no significant change in annual median body weights with time for any of the age/sex groups ($R_s \leq 0.15$; $P \geq 0.37$). Annual median body weights for different sex and age categories were compared using a GLM for years in which data were available for each of the four age/sex categories. Body weight data were ranked prior to analysis, age and sex were factors in the model, year was a random factor, and a term for interaction between age and sex was included in the analysis. Age and sex were the only significant terms in the model ($F_{1,66} > 14.5$, $P<0.001$), indicating females were significantly heavier than males and adults heavier than first year birds. The lack of significance for the age*sex interaction term indicated that the difference in body weight between adults and first years did not vary between females and males.

Our analysis indicated that annual median weight data should be presented separately for the different age and sex categories. Data for adult males and females and first-year females had normal distributions and arithmetic mean and associated 95% prediction intervals were calculated for the control chart (Fig. 6). For first year males, annual median body weight were not normally distributed and the control chart for this group was based on the median value and the prediction intervals were the 1% and 99% percentiles (Fig. 6). Unusual future years would be identified as those in which the annual median body weight fell outside the prediction limits.

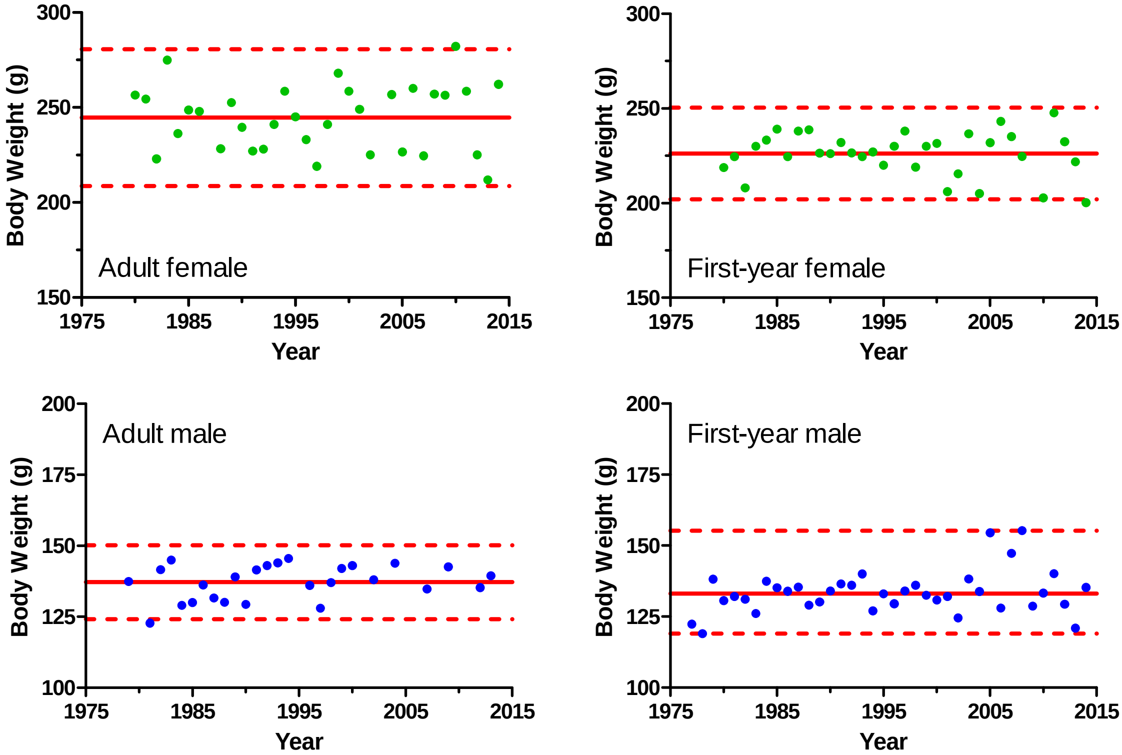


**Figure 6.** Annual median body weights of adult and first-year male and female sparrowhawks where n≥5. For adults and first-year females, the solid line indicates the arithmetic mean value and dotted lines are 95% prediction intervals. For first year males, the solid line represents the median and the dotted lines the 1% and 99% confidence intervals.

## 3.5. Putative Cause of Death

During the PM examination of the birds, a putative cause of death category is assigned to each bird based on a combination of the circumstances in which the bird was found and macroscopic observations of the carcass. Starvation and disease are clumped together into one putative cause, ("starvation or disease") partly because starvation may be a result of disease state, and it is recognised that only gross clinical sign of disease will be identified because post-mortem examination is limited to gross clinical observation. Hereafter, this cause of death is referred to for ease as starvation/disease.

The annual proportion of birds assigned to starvation/disease as a cause of death category were analysed to determine whether temporal trends were evident (Fig. 7). Data were separated by age class and sex and annual sample sizes of < 10 were excluded so that the absolute minimum resolution for proportion data was 10%. Where there were less than 10 birds per year in consecutive years, birds were pooled so that the minimum sample number was ≥ 10 and the data were ascribed to the year that was the mid-point of the data-pooling period. Initial correlation analysis indicated that there was a decline over time in the proportion of adult males that were starved/diseased but no there was no significant change over time or other age/sex classes (P>0.05 in all cases).

Data for all four age/sex categories were arcsine square-root transformed so that the datasets were distributed normally. Means and associated 95% prediction intervals were calculated for adult females and first year males and females. Data for two years (1970 and 1994) were excluded from the dataset for first-year males as the values for both years (zero) were some 4-5 standard deviations from the mean and may have, in fact, represented unusual years. Data for adult males were further investigated by linear regression which confirmed that the proportion of adult male sparrowhawks that were starved declined significantly over time ($R^2$ = 0.221, $F_{(1,29)}$ = 8.25, P=0.0075). The regression model and its associated 95% prediction intervals were then extended to the year 2020 so that the likelihood of the proportions of starved birds in future years, based on current trends, could be assessed (Fig. 7). In this way, "control charts" for all four age/sex class categories of sparrowhawks could be generated (Fig. 7) which would help identify unusual years in the future.

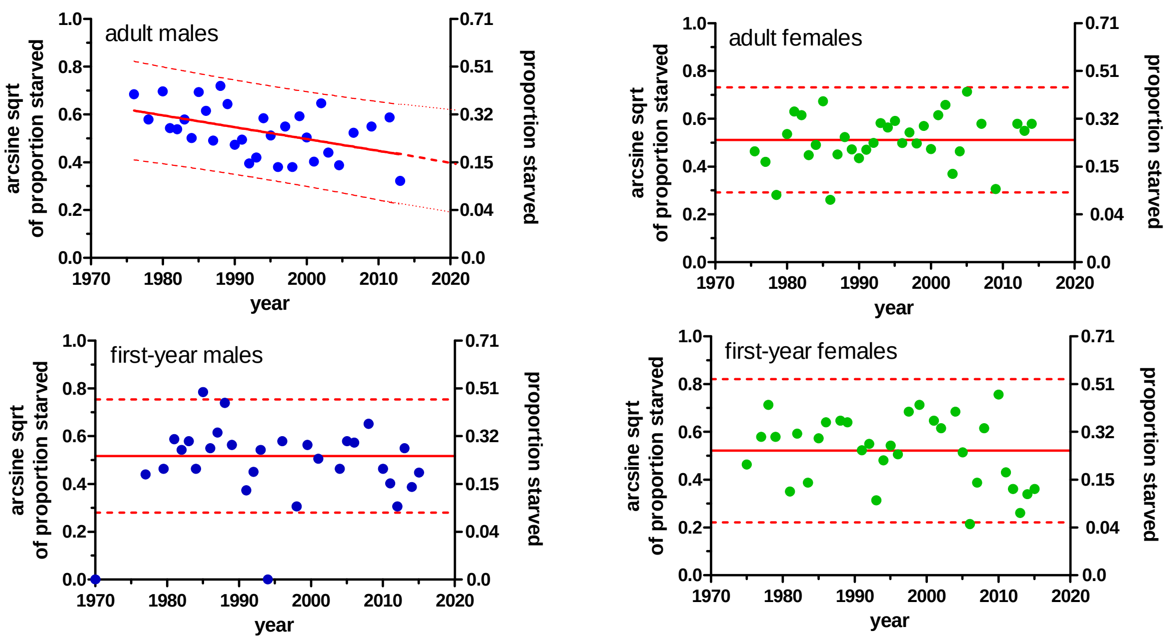


**Figure 7.** The proportion of male and female adult and first year sparrowhawks that had died due to starvation or disease in each year of the monitoring scheme. Except for adult males, the red solid line indicates the mean and 95th percentile prediction interval (dashed red lines); the equivalent lines for adult males are for the linear regression model and its 95% prediction intervals and have been linearly extrapolated forward to 2020.

## 3.6. Fat Score

During the PM examination, a non-linear semi-quantitative categorical fat score is assigned to each bird based on the fat deposits evident in the carcass (Table 1). In very active hunting species such as sparrowhawks, even healthy individuals would rarely have a fat score above 3. For the purposes of the current analysis birds were categorized as starved or non-starved if they had a fat score of 0-1 and 2-5, respectively. This index could give a higher proportion of birds starved than that estimated by cause of death category as it would include birds that have been killed by other causes while they were in a starved state.

**Table 1.** Criteria for assigning a fat score to birds based on fat deposits evident in the body

| Fat Score | Description |
|---|---|
| 0 | No sign of fat deposits within the body including around the heart. |
| 1 | Trace amounts of fat deposits including deposits around the heart. |
| 2 | Small amounts of fat deposits evident, including around the pectoral muscle. |
| 3 | Moderate amount of fat deposits evident, including around the pectoral muscle. |
| 4 | Good amount of fat deposits, including intra-abdominal deposits. |
| 5 | Abundant fat deposits, would be able to recover greater than 2 grams of fat from body. |

Age and sex groups were analysed separately to determine if there was any systematic change over time. Annual sample sizes of < 10 were excluded so that the absolute minimum resolution for proportion data was 10%. There were insufficient data for adult male sparrowhawks for most years and data were pooled for pairs of years staring 1990 and data or pairs were also excluded from the analysis when sample numbers remained <10.

There was no significant correlation between the proportion of starved birds and year for adult females, adult males and first-year females (P>0.22 in all three cases). However, the proportion of first-year males that were starved was positively associated with year (Rs=0.567, P= 0.005), although it was not possible to determine a significant regression model that satisfactorily described the relationship between year and the [arcsine square-root] transform of the proportion data. For adult male, female and first-year females, the proportion data approximated a normal distribution and so arithmetic means across the monitoring period, together with the associated 95% prediction intervals, were calculated (Fig.8)

Overall, therefore, it was possible to establish control charts for this health index except for first-year males. However, the 95$^{th}$ prediction intervals for first-year females and particularly adult males, were wide and suggests that this index may be relatively insensitive as a measure by which to monitor health status of birds submitted to the PBMS. It is unclear why fat scors are so variable but it is likely to reflect variation among individuals in time and cause of death.

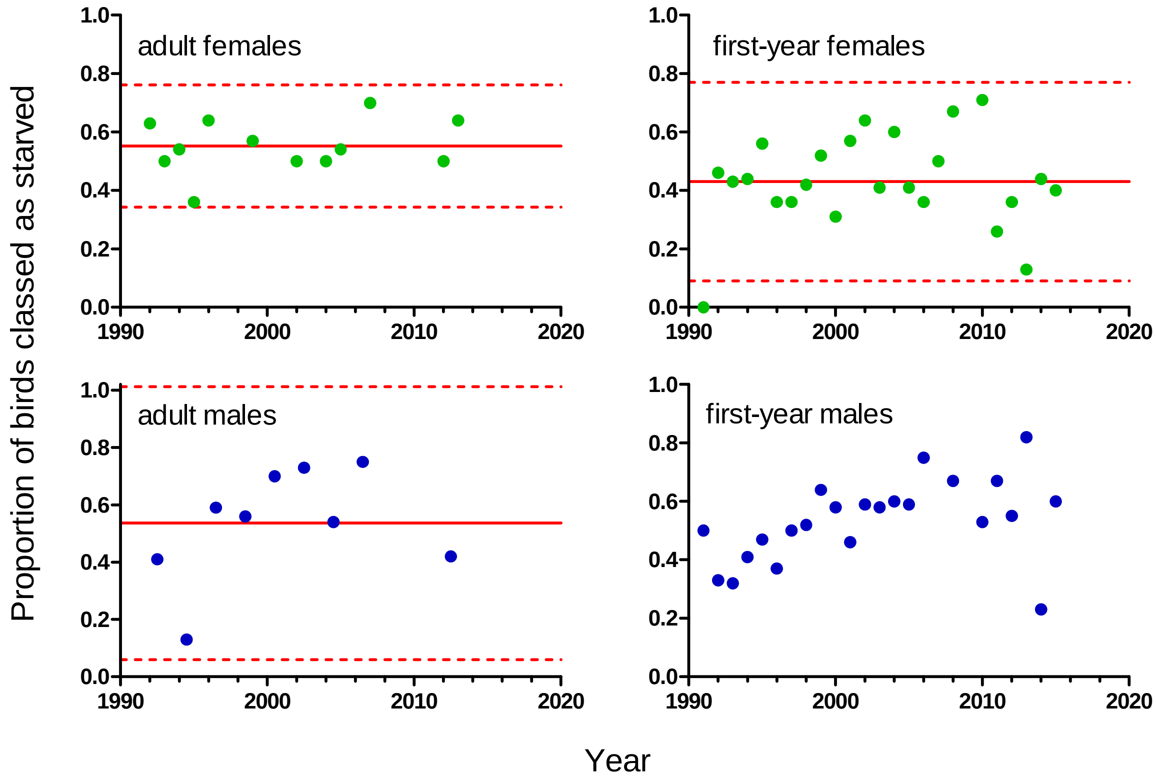


**Figure 8.** The proportion of birds that were categorised as starved, based on their fat score, received by the PBMS per year. Mean and 95$^{th}$ prediction intervals are presented for each group by the solid and dashed red lines, respectively, but are not shown for juvenile males because proportions were positively associated with year.

## 3.7. Condition Index

Since 1975 biometric data for sparrowhawks received by the PBMS has been recorded that allows a quantitative measure of the condition of birds to be calculated. This condition index (CI) is calculated as per equation 1.

Equation 1

$$CI = (Body_{wt} - Gizz_{wt}) \div Sternum\ Diag.^3$$

where

$Body_{wt}$ = Whole body weight (g)

$Gizz_{wt}$ = Wet weight of gizzard contents (g)

Sternum Diag. = Distance between posterior point of the sternum keel plate and the distal point of the clavical (mm)

The CI effectively gives a ratio between the body weight of the bird and an independent measure of the size of the bird. We were unable to fit general linear models to examine if age and year significantly explained variation in the CI as the residuals from the model were not normally distributed irrespective of whether actual values or log transform values were used. We therefore separated the data by sex and age class and used annual mean CI for those years where sample sizes were >5, thereby excluding years where CI data were based on a very small sample of birds. Initial exploration of the data by Spearman rank correlation analysis indicated that there was not a significant relationship between mean annual condition index and year for all age and sex classes ($r_s \leq 0.33$, $P \geq 0.07$) except for adult males ($r_s = 0.61$ P=0003). Further exploration of the data for adult males using linear regression analysis indicated a significant increase in body condition over the monitoring period ($R^2 = 0.50$; $F_{1,20}$ = 2001, P=00002; Figure 9), consistent with the reduction in the proportion of adult males being diagnosed as having died from starvation (Fig. 7).

The annual CI data were normally distributed for all four age/sex classes and so mean (and associated 95% prediction intervals) were calculated for adult females and first-year males and females. The 95% prediction interval for the regression model for adult males was also calculated and the model extrapolated forward to the year 2020, so that it would be possible to examine whether future data is consistent with the trend in CI observed to date.

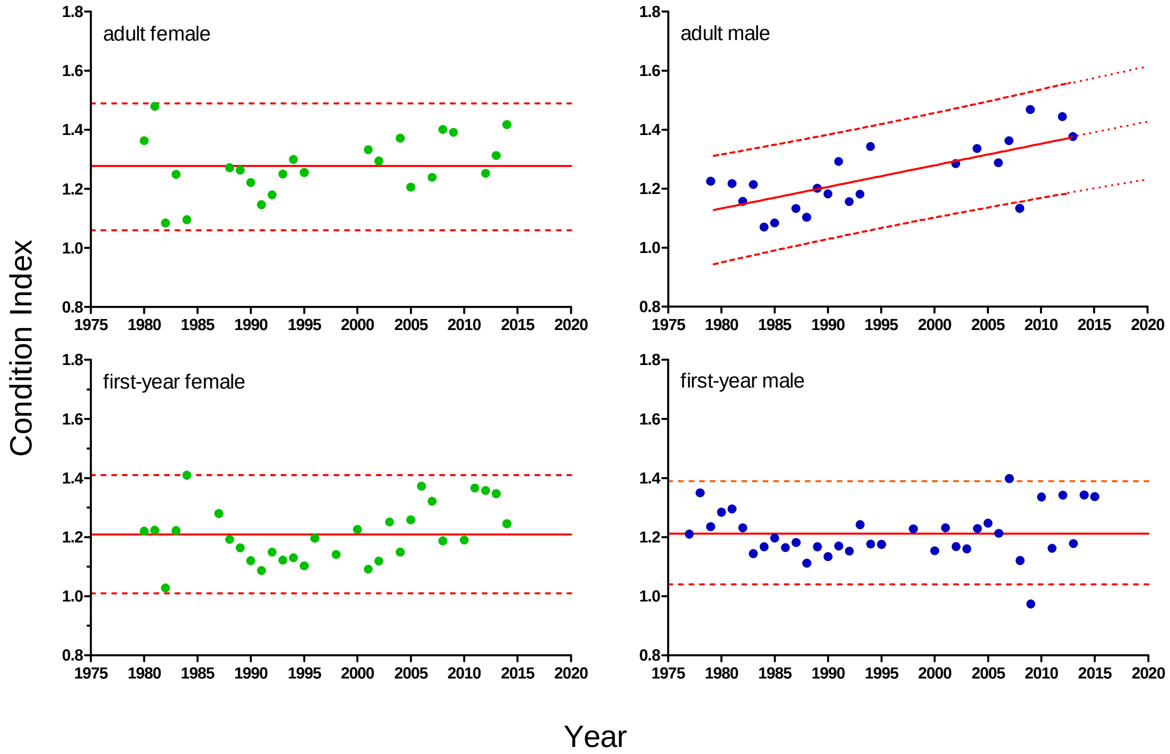


**Figure 9.** Annual mean condition index values for sparrowhawks plotted against year that the bird died. Mean and 95$^{th}$ prediction Intervals are presented for adult females and first-year males and females and are represented by the solid and dashed red lines, respectively. A regression model (and 95% prediction intervals) are presented for adult males

## 3.8. Fluctuating Asymmetry

Fluctuating asymmetry (FA), the random deviation from perfect symmetry in bilaterally paired structures (Tompkins and Andrews, 2001, Palmer and Stroebeck, 1992), has been described as a sensitive and cost-effective indicator of environmental stress (Palmer and Stroebeck, 1986, Leung and Forbes, 1996). It has been shown to increase with environmental stressors such as toxin exposure (Bustnes *et al.*, 2002, Eeva *et al.*, 2000, Mckenzie and Clarke, 1988), adverse temperatures (Chang *et al.*, 2007), nutritional stress (Swaddle *et al.*,

1994) and habitat disturbance (Maul and Farris, 2005, Lens *et al.*, 1999). FA has been described as a measure of developmental stability, — the ability of an organism to achieve its target phenotype (bilateral symmetry) within a particular environment (Palmer, 1994). However, developmental noise can manifest itself as the random perturbations of cellular processes that occur during development (i.e. small random differences in rates of cell division, cell shape formation or cell growth). Such noise opposes developmental stability and therefore increase FA (Klingenberg, 2003, Palmer, 1994, Palmer and Stroebeck, 1986). Developmental noise may be increased by both extrinsic (environmental) and intrinsic (genetic) stress (Palmer, 1994).

In its simplest form, FA is calculated as the difference between the right (R) and left (L) sides (L-R) of a trait (Palmer and Stroebeck, 1986). The trait selected for this study was the 10th primary feather weight. This trait was chosen since other studies had shown these or similar traits correlated positively with environmental stress (Anciaes and Marini, 2000, Brown and Brown, 1999, Bustnes *et al.*, 2002, Eeva *et al.*, 2000) and data were available from the PBMS PM examinations. Fluctuating asymmetry levels may be inflated by measurement error (ME; Palmer & Stroebeck, 2003) therefore quantifying measurement error prior to conducting FA analyses is necessary. To determine ME, two replicate measurements (M1, M2) of the right and left 10th primary feather weight were made from a sub-sample of 30 birds.

For the 10th primary feather weight, the between-sides variation (FA) was greater than the variation due to ME, with ME being 47.5% of the FA. The frequency distributions for this trait, in the entire dataset was normally distributed (KS normality test, KS distance = 0.045, P>0.10) around a mean of zero (Fig. 10) and therefore showed no evidence of anti-symmetry or directional asymmetry.

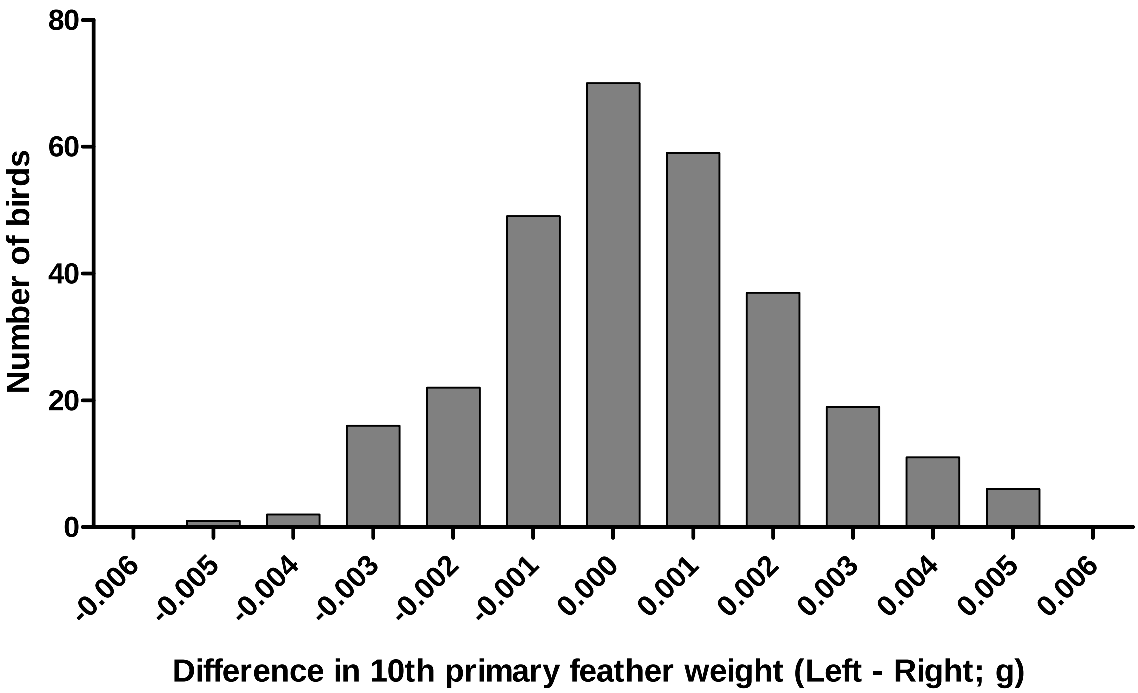


**Figure 10.** Histogram of difference in weight of left and right 10$^{th}$ primary feather in sparrowhawk submitted to the PBMS between 2008 and 2015 inclusively (n = 292).

| Equation 2 |
|---|

$$FA10_w = 0.798 * \sqrt{(|FW_L - FW_R| - ME)}$$

Where
$FW_L$ = Weight of left 10th primary feather (g)
$FW_R$ = Weight of right 10th primary feather (g)
ME = Measurement Error estimated form 2 repeat measures of the trait

A fluctuating asymmetry index for the 10th primary feather weight ($FA10_w$) was calculated (Equation 2) for each bird submitted to the scheme between 2008 and 2015 (Palmer and Stroebeck, 2003); this takes into account ME. General linear model analysis indicated neither age, sex nor the year the bird died were significant factors in predicting the $FA10_w$ index ($F_{1,258} \leq 2.2.03$, $P \geq 0.17$) but there was a significant interaction between age and sex ($F_{1,235} = 5.51$, $P=0.03$); no other interaction terms were significant. The interaction between age and sex was because while the mean (± SD) FA index was similar for adult females (0.029 ± 0.012, n=47), adult males (0.029 ± 0.010; n=33) and first-year females (0.030 ± 0.014; n=105), it was lower in first-year males (0.025 ± 0.011; n=86), although the reason for this is unknown.

This analysis suggested that separate control charts need to be generated at least for first-year males. We used data where the number of birds of each age/sex class in the year was >5, thereby excluding years where CI data were based on a very small sample of birds. This resulted in few data points for adults and data for adult males and females were pooled but data for first-year birds were analysed separately (Fig. 11)

Leung *et al.* (2000) suggests using composite FA index based on multiple traits as this approach may be more likely to detect FA associated with stress. Within the post mortem examination procedures used in the scheme employed the other bilateral traits measured are tarsus length and wing length. However, the precision with which these are measured (to the nearest 0.5mm) is a constraint to using these traits in a composite FA index, because a large proportion of birds show no asymmetry within the measurement error (Table 2).

**Table 2.** Summary of precision of measurements of candidate traits for calculation of fluctuating asymmetry.

| Trait | Precision | Units | n | Percentage with No Asymmetry | Percentage Measurement Error |
|---|---|---|---|---|---|
| 10th Primary feather | 0.0001 | g | 292 | 0.3% | 47.5% |
| Tarsus length | 0.5 | mm | 243 | 89% | Not Estimated |
| Wind length | 0.5 | mm | 244 | 85% | Not Estimated |

Percentage Measurement Error calculated as = (mean ME / mean absolute asymmetry)*100

.

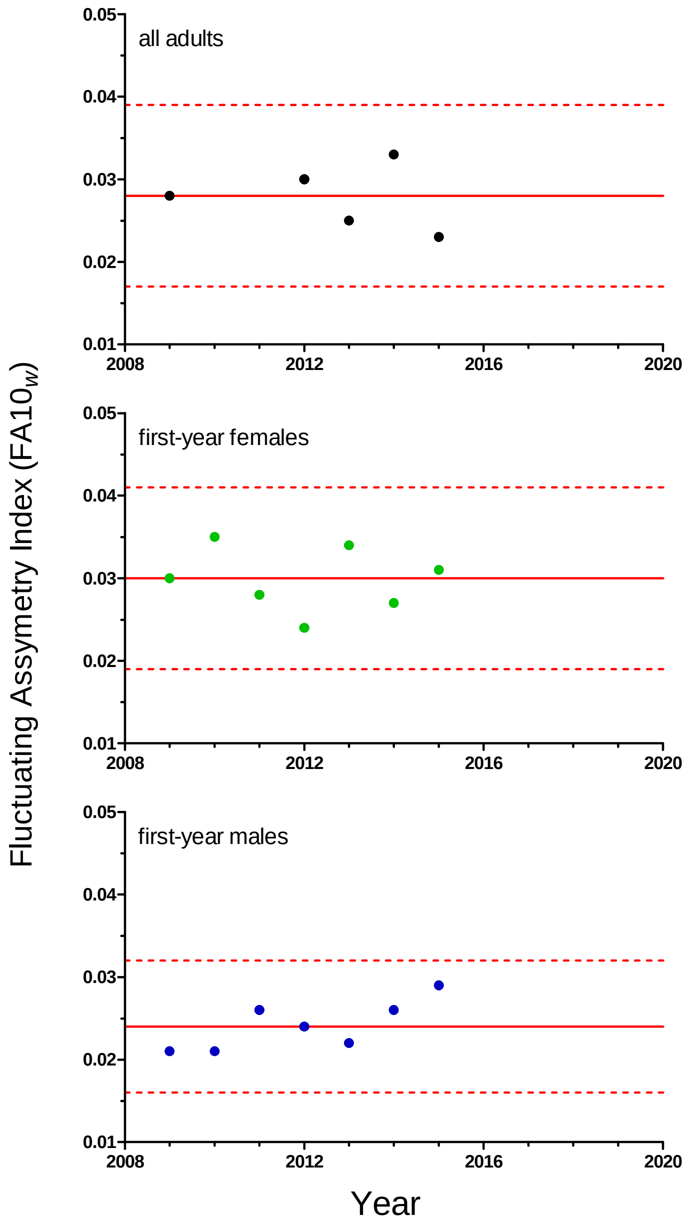


**Figure 11.** Annual mean fluctuating asymmetry (FA) index based on 10th primary feather weight for adult (sexes pooled), first-year female and first-year male sparrowhawks. Mean and 95th prediction intervals are presented by the solid and dashed red lines, respectively.

## 3.9. Eggshell thickness

The effects of exposure to DDT on eggshell thickness and subsequent effects on reproductive success are well-documented (Newton *et al.*, 1982, Newton, 1986, Newton *et al.*, 1986, Newton and Galbraith, 1991, Blus, 2011). Eggs in general are a key resource in ecotoxicological studies (Espin *et al.*, 2016) and shell thinning can be an early warning biomarker of reproductive effects that occurs at exposures that are lower than those that cause direct impacts (Helander *et al.*, 2002).

There are various ways in which to calculate an index of eggshell thickness and it is usually based on measurements of shell mass and egg dimensions (Espin *et al.*, 2016). Eggshell thickness for eggs received by the PBMS are calculated on the basis of equation 3.

Equation 3

$$S.I. = \frac{Wt * 10}{L * B}$$

where
S.I. is Shell Index
Wt. is dry weight of shell (g)
L is length of shell (cm)
B is breadth of shell (cm)

The PBMS has collected data on eggshell thickness in sparrowhawks from the 1960s and also collected data from museum specimens for time periods before the start of the use of DDT. These data clearly illustrate the effect of widespread use of DDT on eggshell thickness from the 1940s and the rate of recovery following voluntary and mandatory bans on use in the 1970s and 1980s (Fig. 12).

We combined data for multiple years when the number of eggs for which we had measurements in each year were sparse. Data for combined years were assigned to the mid-year of the period over which data were pooled and the minimum sample size for which a mean value was calculated was 10 eggs (range 10-118). Data for shell thickness of sparrowhawk eggs collected before the Second World War (which was before any widespread use of DDT in Britain) were used to generate shell index "control charts" for sparrowhawk eggs that could be considered "healthy" in that they were not impacted by DDT. Pre-1939 shell indices were normally distributed and were not significantly correlated with year of collection (Pearson r = -0.354, P=0.12; n = 21), and so mean and 95% prediction interval for shell index were calculated for pre-1939 eggs (Fig. 12).

This analysis suggests that, following the bans on DDT use in Britain and elsewhere, eggshell index in sparrowhawks recovered to pre-1939 levels during the 1990s and there may have been some type of "rebound" in that, in some years post-2000, shell indices exceeded the 95% upper prediction interval (Fig 12.)

This analysis suggests that shell index can be used as an indicator of reproductive health in sparrowhawks that may provide early warning of potential adverse impacts on reproductive potential.

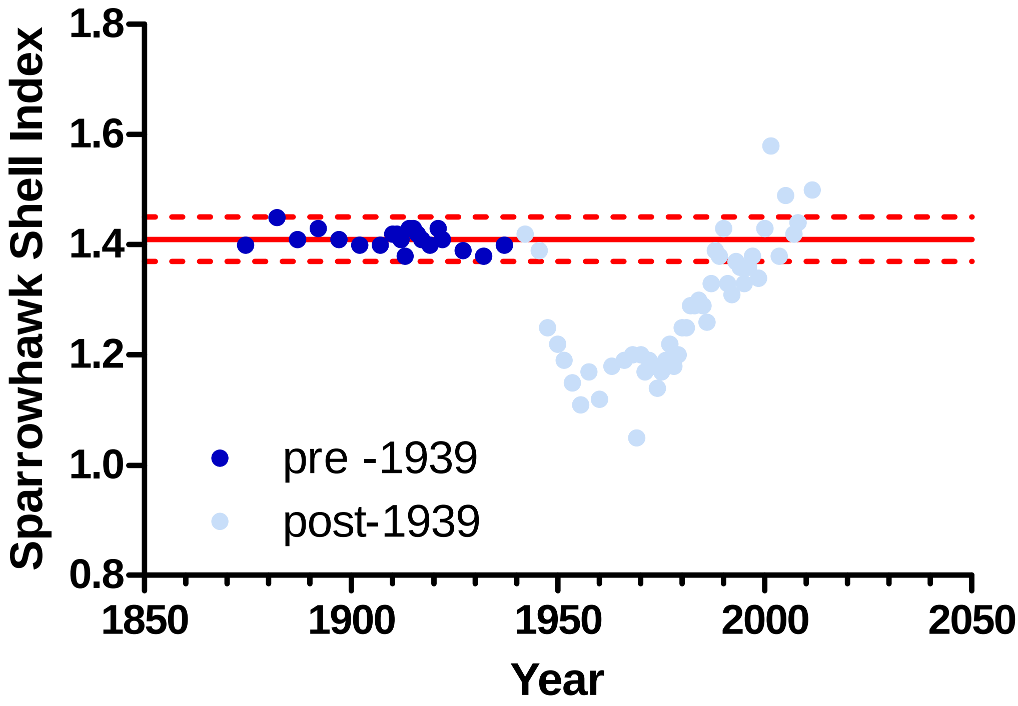


**Figure 12.** Mean eggshell thickness index for sparrowhawk eggs dating from before and after 1939. Mean and 95th prediction intervals, based on pre-1939 data, are presented by the solid and dashed red lines, respectively.

## 3.10. Hormone levels in feathers

A study of feather corticosterone (CORT) content in predatory birds was carried out using feathers collected by the PBMS (Strong *et al.*, 2015). The study investigated the feasibility of measuring corticosterone in feathers from PBMS specimens to provide a retrospective assessment of the activity of the stress axis (hormonal releases in response to stress) in relation to contaminant burden. Feather samples were taken from sparrowhawk, *Accipiter nisus*, kestrel, *Falco tinnunculus*, buzzard, *Buteo buteo*, barn owl *Tyto alba*, and tawny owl, *Strix aluco,* and the variation in feather CORT concentrations with respect to species, age, sex, feather position, and body condition was assessed. In sparrowhawks only, variation in feather CORT content was compared with hepatic metal concentrations as a one-off study; feather metal concentrations are not routinely measured as part of the PBMS monitoring programme.

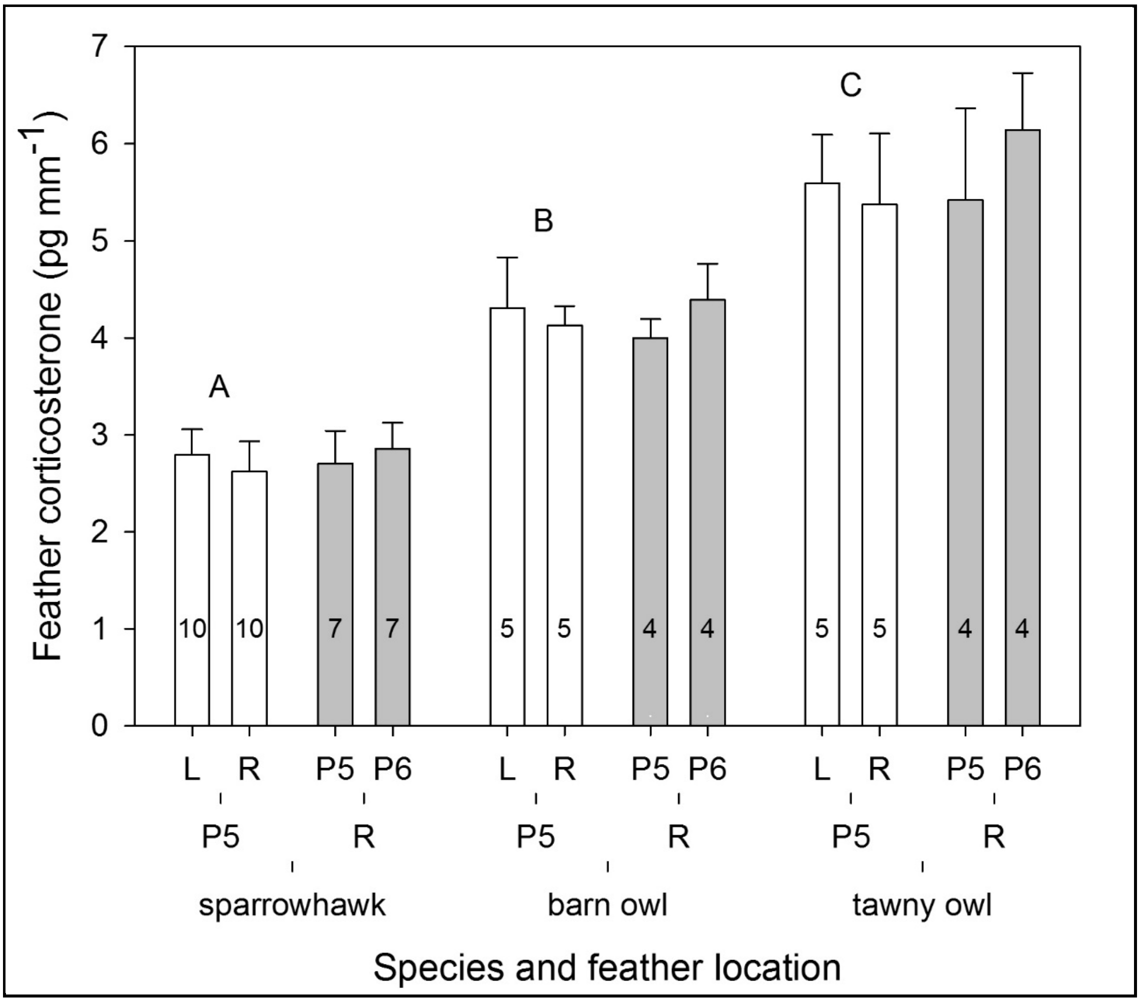


**Figure 13.** Comparison of corticosterone concentrations in primary flight feather (P5) from left and ring wings (L and R) and for adjacent primary flight feather (P5 and P6) within the right wing (R) in three species of raptor. Each bar is the mean + SEM, with n values indicated inside the bars. No significant differences were apparent between P5L and P5R or between P5R and P6R within species. Significant differences between P5 CORT content across species are indicated by dissimilar letters (Figure taken from Strong et al., 2015).

For individuals, CORT concentration (pg $mm^{-1}$) in adjacent primary flight feathers (P5 and P6), and left and right wing primaries (P5), was statistically indistinguishable (Figure 13). The lowest concentrations of CORT were found in sparrowhawk feathers and CORT concentrations did not vary systematically with age or sex for any species (Figure 14). In sparrowhawks, feather CORT concentration was found to be positively related to the hepatic concentrations of five metals (cadmium, manganese, cobalt, copper, molybdenum) and the metalloid arsenic (Figure 15). The results suggested that some factors affecting CORT uptake by feathers remain to be resolved but feather CORT content from archived specimens has the potential to provide a simple effects biomarker for exposure to environmental contaminants.

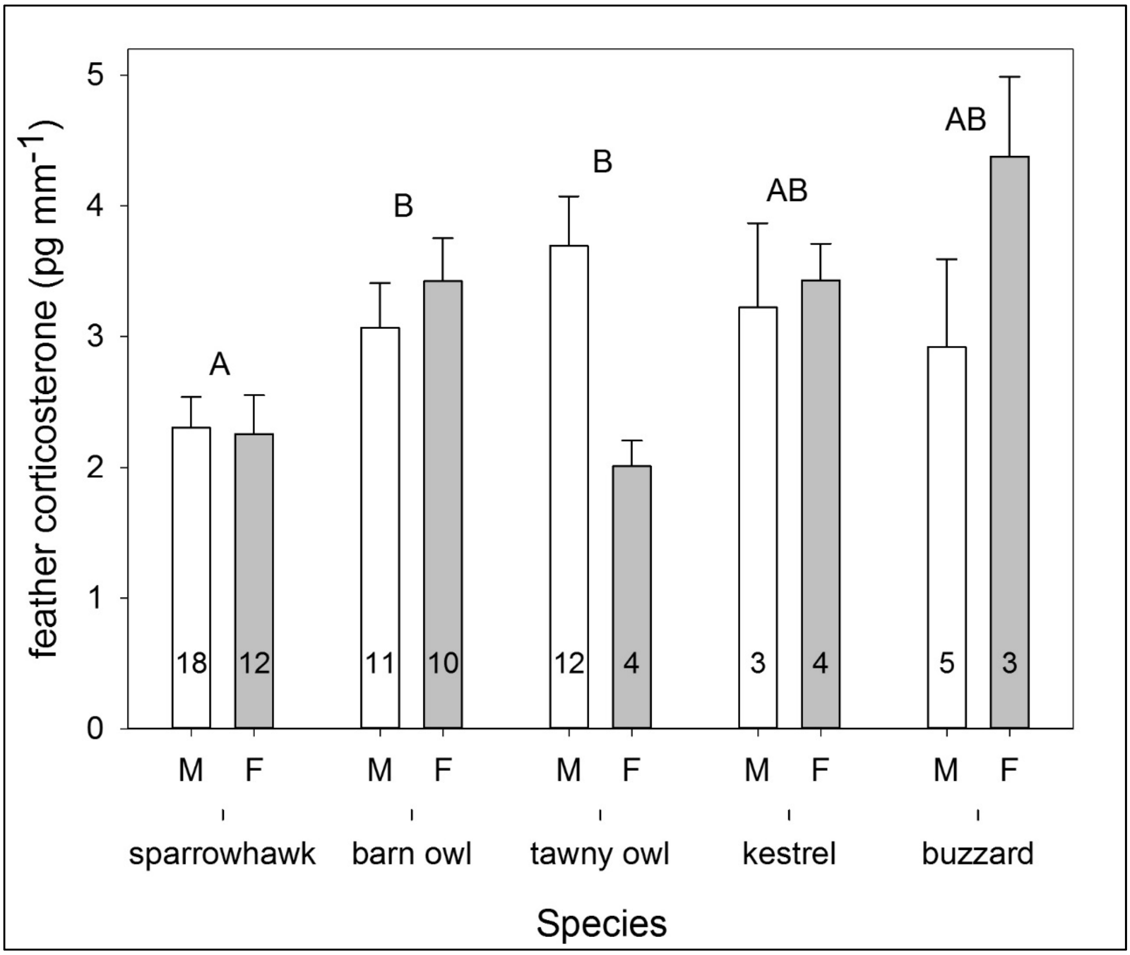


**Figure 14.** Mean feather concentrations of CORT in the P5 flight feathers of five raptor species. Each bar is the mean + SEM. Males (M) and females (F) are shown separately for each species and n is indicated by the number within the bar. No significant differences between sex within species were observed, letters above each species pair denote differences between species overall. Means sharing the same letter are not significantly (Figure taken from Strong et al., 2015).

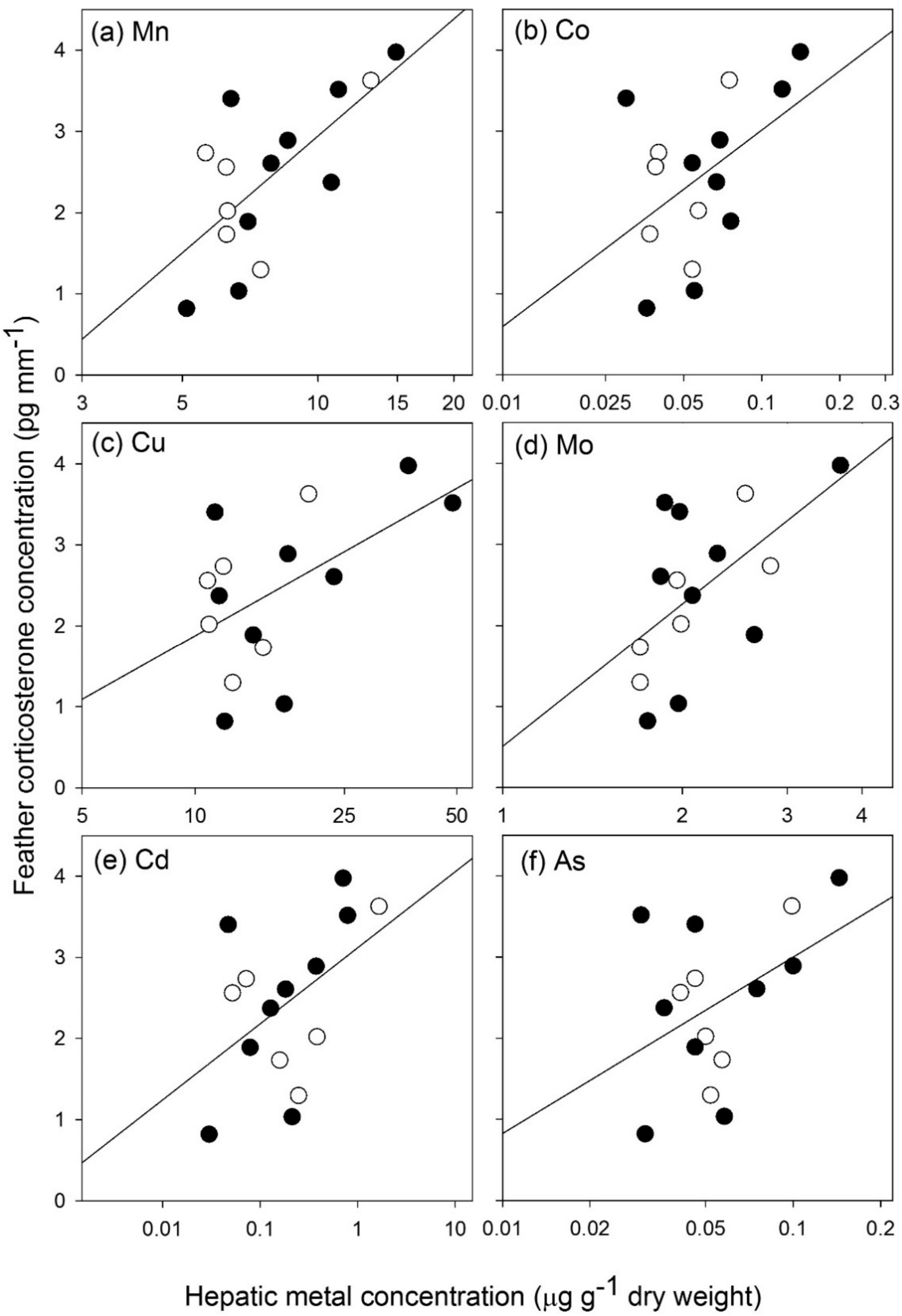


**Figure 15.** The relationship between feather corticosterone concentration and liver metal concentration in fifteen sparrowhawks. The linear regression best-fit line is shown for each metal; (a) Mn: $r^2 = 0.48$, $P = 0.004$; (b) Co: $r^2 = 0.31$, $P = 0.03$; (c) Cu: $r^2 = 0.3$, $P = 0.03$; (d) Mo: $r^2 = 0.32$, $P = 0.03$; (e) Cd: $r^2 = 0.31$, $P = 0.03$; (f) As: $r^2 = 0.26$, $P = 0.05$. Results for male birds are denoted by filled circles, females by unfilled circles (Figure taken from Strong et al., 2015).

Recommendations from the Strong et al (2003) study were that:

- For within-species comparisons, care should be taken to compare feathers matched for size.
- Comparisons of feather CORT concentrations among age and sex classes were based on relatively low sample numbers (≤18 birds) and so effects of these factors may be under-estimated.
- Feather CORT showed significant trends in relation to month of death (declining during the year) and year (rising successive years) and so causes of these observations should be investigated. For example the increasing CORT concentrations in successive years may reflect an increase in stress levels experienced by those birds or a decline in CORT levels during storage.
- Further investigation of the links between metal loading and feather CORT concentration are justified, particularly regarding the possibility of a functional relationship between the two. In particular, the simultaneous measurement of both contaminant concentrations and CORT in feathers that are shed serially, together with analysis of contaminant levels in tissues that bioaccumulate pollutants, would help considerably in establishing the relationship between feather steroid content and its functional significance.

# 4. Conclusions

Several of the candidate indices investigated in this report would appear to be suitable for developing an annually updated population health index based on a classic quality control chart approach. In some cases, changes in the metrics can be broken down such that variation with age class and sex can be accounted for, thereby providing greater potential insight as to what changes may be occurring and their potential demographic effect.

A summary of the potential health indices, together with their likely reporting frequency and what type of change they may indicate, is given in Table 3. These indices provide information about potential change in what can be broadly grouped as three categories: (i) population demography because of altered recruitment, survival and mortality; (ii) nutritional status; and (iii) physiological stress. The measurements necessary to calculate these indices are routinely captured by the PBMS through direct input into an Oracle database at the time of PM examination. Health index reporting would therefore not require extra annual resource and would help maximise the value of information currently captured.

Of the metrics listed in Table 3, some are more sensitive or objective than others. Metrics based on reporting of a proportion have the disadvantage that it is not necessarily possible to determine if any change is due to variation in the numerator in the proportion, the denominator or both. We have also explored a number of potential indicators of nutritional status of the birds submitted to the PBMS. Measures based on body weight do not account for change in body size while those based on fat score are categorical and somewhat subjective. Condition index is derived solely from empirical measurement and accounts for body size. While this may make condition index seem to be the most robust and potentially sensitive single measure amongst those considered, it is not necessarily sensitive to, for example, change in body size. Consideration of changes in all three indices (together with information on the proportion of birds diagnosed to have died from starvation) would probably give a clearer indication of change in nutritional state over time.

The proposed health index of fluctuating asymmetry based on the 10$^{th}$ primary feather weight is a measure related to environmental stress during the period of feather development rather than at the time the death of the bird. It would therefore be important to link any change in this metric to changes in other metrics for the year in which moult was likely to have occurred.

Of the metrics considered in the current report, Table 3 does not include number of birds submitted to the PBMS or feather hormones. With regards numbers, reporting of the data is simple and would of necessity be provided alongside any health index metrics. However, its use as an index in its own right is questionable. This is because our analysis suggests that the previously observed relationship between variation in numbers submitted to the PBMS and variation in population size no longer holds true. In that respect, overall health index reporting would be better served by referencing BTO population trend data to the indices listed in Table 3. With regards feather hormone measurement, production of a health index based on such measurements is entirely possible but would require considerable additional resource in the same way as needed for annual measurements of pollutants in carcasses or feathers (to provide information on exposure and possible effect) or annual measurements of feather stable isotope composition (which can provide a potential measure indicative of dietary change). While it could be argued that all three would be desirable, their expense is likely to mean that such measurements would normally be conducted as part of a specific study.

**Table 3.** Possible metrics gathered during PM examination that can be used as indicators of health status

| Metric | General Indicator Category | Possible indicator of change in: | Demographic group | Potential reporting frequency | Limitations |
|---|---|---|---|---|---|
| Sex ratio | Recruitment and survival | Relative recruitment or survival of males and female | Adults and first-year birds pooled | Annual, depending on sample size | May not reflect true sex ratios but indicative of change within sampling structure |
| Proportion of first year birds | Recruitment and survival | Recruitment success relative to adult numbers and/or change in relative mortality of adult and/or first year birds | | Annual, depending on sample size | Unclear whether indicates change in first-years or adults |
| Eggshell index | Recruitment and survival | Nutrition, exposure to pollutants, or other factors that affect eggshell production | Adult females | Annual, depending on sample size | May not be sensitive metric for many stressors |
| Proportion deaths from starvation or disease | Mortality | Change in relative frequency of major causes of death. Possible indicator of change in nutritional status | Adults and first-year birds separately | Annual, depending on sample size | Unclear whether reflects change in relative numbers dying from this or other causes |
| Body weight | Nutritional status | Food availability/quality or other factors affecting nutritional status | Adults and first-year birds separately | Annual, depending on sample size | Does not account for change in body size. Use in conjunction with other measures of nutrition |
| Fat score | Nutritional status | Food availability/quality or other factors affecting nutritional status | Adults and first-year birds separately | Annual, depending on sample size | Categorical and subjective score. Use in conjunction with other measures of nutrition |
| Condition index | Nutritional status | Food availability/quality or other factors affecting nutritional status | Adults and first-year birds separately | Annual, depending on sample size | Does not account for change in body size. Use in conjunction with other measures of nutrition |
| Fluctuating asymmetry | Physiological stress | Change in stress levels in general populations | Adults and first-year birds separately | Annual, depending on sample size | Indicative of stress at time of feather growth but not at time of death |

One salient question is how any health indicators would be reported by the PBMS. It is proposed that, given all the measurements are directly entered into an Oracle database during the PM examination, these metrics could be calculated immediately and either status updated at the end of each calendar year or even live reported during the year as data are collected and immediately made available through the PBMS website.

There are a number of ways in which data could be presented. One option could be that data are presented in the form of control charts as illustrated in the current report, although they could be modified, potentially using 90% and 95% prediction intervals to give a more graded response of unusual deviation away from the annual average. Any such charts could be embedded behind a simple traffic light display in which, for example, green would indicate the annual average is within the 90$^{th}$ percentile prediction interval, amber indicates it falls between the 90$^{th}$ and 95$^{th}$, and red indicates the annual average is out with the 95% interval. Traffic lights could be provided for each metric and compiled as a three-compartment dashboard reflecting the broad three categories outlined in Table 3. Numerous variations on this theme would be possible.

This study to date has focused on just one of the core species collected by the PBMS, the sparrowhawk, as a proof of concept. It is envisaged that similar health indices metrics could be compiled for all the core species which are collected by the PBMS in sufficient numbers, enabling health indices to be compiled for different trophic strategists (predators of small mammals, predators of birds, scavengers) in the terrestrial environment. It may also be possible that such an approach could be extended to the Cardiff University Otter Project, one of the PBMS partner organisations in the WILDCOMS network, thereby providing real-time health index surveillance for sentinel species in both the terrestrial and freshwater environments. Similarly, provided annual sample sizes are of sufficient size, it is likely to be possible to report health index metrics for animals collected from specific regions or countries within the UK that may vary in their conservation policies or uptake of agri-environment or other schemes.

# 5. Acknowledgements

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