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Investigating supplementary food use of UK Hawfinch *Coccothraustes coccothraustes* populations using DNA metabarcoding

Ewan H. Stenhouse [a], Will B. Kirby [b], William Bernard Perry [a], Angela Marchbank [a], Trudy Workman [a], Paul Bellamy [b], Ian P. Vaughan [a], William O.C. Symondson [a] and Pablo Orozco-terWengel [a]

[a]School of Biosciences, Cardiff University, Cardiff, UK; [b]RSPB Centre for Conservation Science, The Lodge, Sandy, UK

ABSTRACT

Capsule: This pilot study showed that DNA metabarcoding can detect supplementary food in the diet of Hawfinches, which differed spatially and between years but not between sexes.

Aims: To explore the potential of DNA metabarcoding to assess the use of supplementary food by Hawfinches, which was supplied experimentally in different woodland sites, and how this varied over space and time.

Methods: We identified supplementary food in faecal samples using DNA metabarcoding techniques. Faecal samples were collected in the 2016 to 2019 breeding seasons at 11 field sites across five UK regions. Prevalence of supplementary food within the diet was then compared between populations and sexes.

Results: Across 286 Hawfinches captured, sunflower *Helianthus* spp. was detected in 30.5% of samples, with significant differences between regions, sites and years, but not between sexes.

Conclusion: DNA metabarcoding was successful in detecting supplementary food in Hawfinch faecal samples, supporting its potential for future studies. Sample sizes from populations were modest and further work would be beneficial to explore how the use of supplementary food changes temporally, phenologically and in relation to natural and non-natural food availability in the wider landscape. Our study highlights the necessity to monitor supplementary food use within a conservation management setting to avoid negative impacts, such as the spreading of diseases.

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Supplementary feeding of birds is an extremely common global form of human-wildlife interaction, estimated to be worth billions of pounds sterling worldwide (Cox & Gaston 2018, Shutt & Lees 2021). It is estimated that 48% of British households provide supplementary food to wild birds (Hanmer *et al.* 2017). Provisioning of supplementary food has increased from winter-only feeding to year-round, on the basis that nutritional demands differ temporally, and therefore are not limited to the 'hungry-gap' months (Siriwardena *et al.* 2008, Lawson *et al.* 2018). Despite garden feeding being actively encouraged by many conservation organizations, the effects on the avian community of this colossal resource addition have attracted limited research interest (Galbraith *et al.* 2015, Plummer *et al.* 2019, Shutt & Lees 2021).

Research exploring the direct impacts of supplementary feeding have focused on population effects, changes to body condition, survival and reproductive success (Siriwardena *et al.* 2007, Plummer *et al.* 2013, Fischer & Miller 2015, Lawson *et al.* 2018, Plummer *et al.* 2018). This research has, however, resulted in contradicting conclusions; some studies have shown supplementary feeding has advanced breeding phenology and improved reproductive success (Peach *et al.* 2014), but others have shown the opposite, potentially due to the supplementary food provided being sub-optimal (Plummer *et al.* 2018). There has also been directly contrasting research with regard to avian health, with some studies showing benefits (Knutie 2020) while others show negative impacts (Plummer *et al.* 2013).

The risks associated with supplementary feeding include the possibility that birds become reliant on artificial food sources or are at higher risk of predation at provisioning sites (Lawson *et al.* 2018). The risk of inter- and intraspecific disease transmission at feed sites is also increased, due to the

CONTACT Ewan H. Stenhouse stenhouseeh@cardiff.ac.uk

long-term and unnaturally high-density aggregations of birds (Lawson *et al.* 2018, Moyers *et al.* 2018). Additionally, competition at feeding sites may result in increased stress and secondary immunosuppression, due to resources being apportioned through hierarchical dominance (Murray *et al.* 2016, Lawson *et al.* 2018). Females and juveniles occur lower in the dominance hierarchy and, therefore, can be out-competed (Hanmer *et al.* 2022). This may result in adult males consuming greater volumes of supplementary feed, increasing their risk of predation or disease transmission (Schaper *et al.* 2021, Hanmer *et al.* 2022). Reproductive success may also be impacted, as supplementary food of low nutritional value can impact sperm quality (Støstad *et al.* 2019).

Research on avian supplementary feeding has tended to focus on species that frequently visit garden feeders, while the effects on rarer species have yet to be fully explored (Peach *et al.* 2014, Hanmer *et al.* 2017, Shutt *et al.* 2021). To date, we know of only one study that has used DNA metabarcoding to explore avian supplementary food use, which focused on a common European species, the Blue Tit *Cyanistes caeruleus* (Shutt *et al.* 2021).

The Hawfinch *Coccothraustes coccothraustes* is a woodland specialist that has been declining in the UK since the 1970s, and has been Red-listed within the Birds of Conservation Concern (BoCC) review since 2009 (Kirby *et al.* 2015, Kirby *et al.* 2018, Stenhouse *et al.* 2023b). A study by Stenhouse *et al.* (2023b) showed that British Hawfinches have a wide dietary niche breadth, feeding on a range of plant and invertebrate taxa. It was suggested by Stenhouse *et al.* (2023b) that Hawfinches may have a core and secondary diet, with the latter playing a role in dietary completion by enabling survival when preferred (or essential) food items are scarce (Tournayre *et al.* 2021, Stenhouse *et al.* 2023b). Studies of Hawfinch populations across Denmark and Germany showed similar results, with supplementary food frequently being consumed, possibly due to Hawfinches being a euryphagic species that benefits from the supply of a continuous and abundant food resource (Palacio 2020, Stenhouse *et al.* 2023a).

Provisioning of supplementary food resources can result in unnatural aggregations of avian species, facilitating novel species interactions (Lawson *et al.* 2018, Moyers *et al.* 2018, Shutt & Lees 2021). A well-known example is the spread of the parasite *Trichomonas gallinae*, which likely spread from Columbiformes to Passeriformes in 2005 (Robinson *et al.* 2010, Lawson *et al.* 2011). This has resulted in the disease trichomonosis rapidly spreading among feeder-using finches, particularly the European Greenfinch *Chloris chloris* and Eurasian Chaffinch *Fringilla coelebs* (Robinson *et al.* 2010). It is estimated that trichomonosis has caused the UK population of European Greenfinches to decline by 66% over a 10-year period, with Eurasian Chaffinches now experiencing a similar decline (Robinson *et al.* 2010, Hanmer *et al.* 2022).

Supplementary feeding beyond the garden context is a tool with many conservation management applications (Hanmer *et al.* 2022). As Hawfinches must adapt to local habitats and resources to satisfy their energy and dietary demands, they select which foraging areas to visit more frequently (Molokwu *et al.* 2011). In areas where natural food is of low abundance, quality, or both, supplementary food use by Hawfinches will be more frequent. Subsequently, increased supplementary food use may lead to localized disease outbreaks or reduced breeding success, contributing to local population declines (Lawson *et al.* 2018). Considering the risks of supplementary feeding, coupled with the Hawfinch being Red-listed, we undertook a pilot study exploring supplementary food use in Hawfinches, which is timely and impactful when considering the conservation measures to mitigate the Hawfinch's decline.

This pilot study builds on results generated by Stenhouse *et al.* (2023b). Specifically, we focused on the use of supplementary food by Hawfinches using dietary data obtained through DNA metabarcoding and high-throughput sequencing, as previously detailed in Stenhouse *et al.* (2023b). We investigated the frequency of occurrence in the diet of UK Hawfinches and expected that supplementary feeding prevalence would differ between distinct populations, sexes and across years. These differences were expected to be consistent with switches in foraging behaviour driven by the availability of natural food resources, different foraging behaviours and nutritional demands of both sexes during the breeding season, and also hierarchical dominance. A goal of this pilot study was to enable the development of further research into the impacts of supplementary food use by Hawfinches and other birds of conservation concern.

Methods

Full details of sampling, DNA extraction, sequencing and bioinformatic methods can be found in Stenhouse *et al.* (2023b). In short, fieldwork was conducted between March and July in the years 2016 to 2019 at

11 woodland feeding sites in the UK. All sites were baited ad libitum from December to July every year, and were pre-existing study areas for catching and ringing Hawfinches within the Wye Valley region (sites: Cinderford, Chepstow, Monmouth and Tintern), the North Wales region (sites: Bontnewydd, Dolgellau, Llanellytd and Penmaenpool), and also north Cardiff (south Wales), the New Forest (southern England), and East Anglia (eastern England). Sites in north Cardiff, the Wye Valley and North Wales were predominantly composed of Common Beech *Fagus sylvatica*, oak *Quercus* spp., and Common Ash *Fraxinus excelsior*. The East Anglia site was a mixed woodland consisting of lime *Tilia* spp., Common Ash and maples *Acer* spp. The New Forest site was dominated by oaks, with an understorey flora comprising of holly *Ilex* spp. and bramble *Rubus* spp. Full site locations are not given for anonymity.

Across all sites, we only used sunflower *Helianthus* spp. seeds as artificial bait to attract Hawfinches, which were trapped using mist nets or whoosh nets. No other supplementary food was used (e.g. mealworms). We placed captured Hawfinches in brown paper bags within cloth bird bags to minimize contamination, and held them for up to 20 minutes, with all birds being processed and released after this time regardless of whether a faecal sample was deposited. We collected individual faecal samples using a new, sterile plastic toothpick each time to avoid contamination. Samples were frozen at −20°C between one to three hours after collection. We extracted DNA from the faecal samples using a Qiagen QIAmp Stool Mini Kit (QIAGEN N.V., Hilden, Germany). We amplified extracted DNA using universal primers UniplantF, 5′-TGTGAATTGCARRATYCMG-3′ and UniPlantR 5′-CCCGHYTGAYYTGRGGTCDC-3′ to amplify a 187–387-bp fragment covering the ITS2 region of plant nuclear DNA (Moorhouse-Gann *et al.* 2018).

For sequencing, we labelled all primers with Multiple Identifier (MID)-tags to label each sample with a unique combination of tags for subsequent identification in downstream analysis. We undertook library preparation using a NEXTflex Rapid DNA-Seq kit (Bioo Scientific, Austin, USA), following the manufacturer's protocol. We sequenced libraries on a V2 chip with 2×250 bp paired end reads on an Illumina MiSeq sequencer (Illumina). We undertook bioinformatic analysis following Drake *et al.* (2021) and Davies *et al.* (2022), with a full description given in Stenhouse *et al.* (2023b).

Statistical analysis

To assess the presence and prevalence of supplementary food in the Hawfinches' diet, we calculated the frequency of occurrence (FOO) by totalling the number of instances that sunflower seeds occurred across all Hawfinch samples. We then calculated this as a percentage of the total number of samples (% FOO) by dividing the frequency of occurrence by the total number of faecal samples collected, and multiplying by 100.

We undertook modelling in R version 4.2.2 (R Core Team 2020) using the binary data presence or absence of sunflower seeds. While individual sites were used within the analysis, to maximize statistical power we also grouped the four individual sites within each of the sampling regions of the Wye Valley region and the North Wales region. The north Cardiff, New Forest and East Anglia regions only contained one site each. There were eleven instances where the same Hawfinch was recaptured. To make these instances comparable with the rest of the dataset, the data from one of the two captures were randomly selected using the 'dplyr' package (Wickham *et al.* 2023). Data included here were only collected from adult birds, due to a small sample of juvenile birds.

North Cardiff and East Anglia were excluded from the modelling due to small sample sizes, and so the final model included samples from the New Forest, North Wales and Wye Valley sampling regions. A generalized linear mixed-effects model (GLMM) with a binomial probability distribution was run using the R package 'lme4' (Bates *et al.* 2015) to understand the effect of variables influencing the binary presence or absence of sunflower seeds in the diet of Hawfinches. Explanatory terms in the model included the region, sampling site nested within its region, sex and capture year (as a categorical variable). The random effect was date, nested within the capture year. Using this GLMM, an analysis of deviance test was conducted with a chi-squared test to assess the statistical significance of terms.

Results

Of the 286 Hawfinches captured, sunflower seeds were detected in 30.5% (%FOO) of the samples and across all study regions (Figure 1(a)). Furthermore, there were significant differences in sunflower presence between years in North Wales and the Wye Valley ($P = 0.03$; Figure 1(b)). Furthermore, the frequency of occurrence of sunflower seed was higher in North Wales than in the Wye Valley. Using a generalized linear mixed model and an analysis of deviance implementing a chi-squared test, the presence of sunflower seed was significantly different between sites (nested within region) (χ^2 (7239) = 18.19, $P = 0.006$),

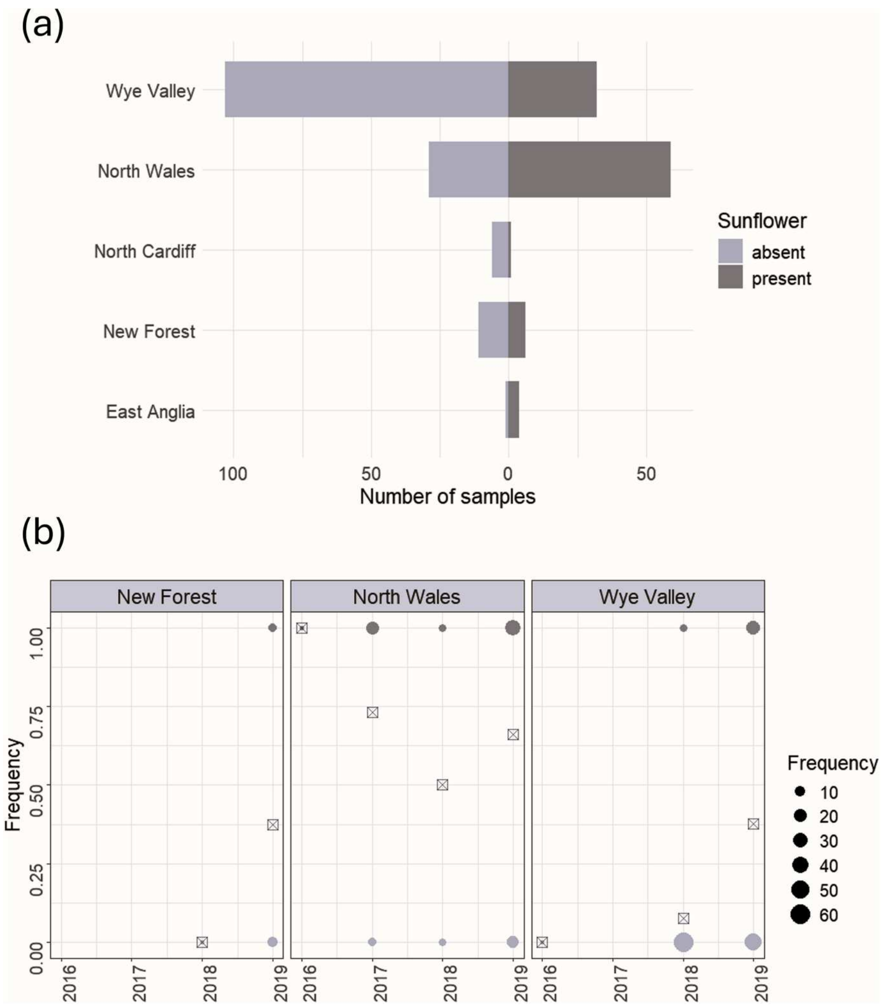

Figure 1. (a) Presence and absence of sunflower seeds in the diet of Hawfinches as a frequency of faecal samples from birds caught in each region; and (b) presence of sunflower seeds in the diet over time in the New Forest, North Wales and Wye Valley sampling regions, where the frequency is indicated by the size of the points. Proportion of sunflower seeds present in the samples is also shown with crossed squares.

year (χ^2 (4239) = 8.92, $P = 0.03$), and regions (χ^2 (7239) = 16.02, $P = < 0.001$). There was, however, no significant effect of sex (χ^2 (3,239) = 0.84, $P = 0.35$).

Discussion

This study revealed that sunflower was the second-most detected dietary item in the Hawfinch faecal samples (a %FOO of 30.5% across all samples) and was present in Hawfinch faecal samples at all sites. No other supplementary food was detected, such as peanuts *Arachis* spp. For comparison, the most frequently detected natural food resource was Common Beech, present in 38.5% of the same samples. This study revealed significant spatial differences at the local and regional scales, as well as between sampling years, but no difference in supplementary food use was found between sexes.

The absence of other supplementary food, such as peanuts, indicates that Hawfinches may not be utilizing such provisions available in the wider landscape. This may be linked to the timing of sampling during the breeding season. For altricial bird species that provision their nestlings, access to high-quality food can impact offspring survival by enabling earlier fledging and settlement on high-quality territories (Harrison *et al.* 2010, Senécal *et al.* 2021). Furthermore, the foraging range may be restricted by the birds' need to provision their offspring. This may result in Hawfinches feeding on more abundant and predictable food items within their immediate foraging environment, as seen in other passerines during the breeding season (da Silva *et al.* 2020).

The frequent detection of sunflower within samples was expected, as all sites were baited ad libitum from December–July every year, with all faecal samples collected within those months. Despite this, Hawfinch populations in North Wales showed the highest prevalence (50.4%) of sunflower within their diet. Previous observations noted that Hawfinch populations in North Wales frequently visit supplementary bird feeders within gardens, and have done for many years (P. Bellamy, pers. obs.).

Landscape features may be a driver of the large amount of supplementary food revealed within Hawfinch populations in North Wales, as human impact on landscapes can directly impact the quality and quantity of resources, influencing diet (O'Hanlon *et al.* 2020, Palacio 2020). This occurs via human-impacted landscapes having reduced or degraded foraging habitat, thus impacting the quantity and quality of consumed resources (White 2008, O'Hanlon *et al.* 2020). European Hornbeam *Carpinus betulus* and Common Beech are considered the main natural food resources for Hawfinch populations in North Wales (Stenhouse *et al.* 2023b). Therefore, it can be suggested that human impacts on the landscape (such as changes in forestry management, or tree species planted) may result in these natural food resources being of limited availability. Hawfinch populations in North Wales may, therefore, be having to feed on supplementary food more frequently to satisfy their energy demands.

In contrast to North Wales, the frequency of occurrence of supplementary food in Hawfinch populations from the Wye Valley was much lower (11.2%), despite a similar number of birds being sampled (North Wales $n = 115$, Wye Valley $n = 138$). This may be due to a higher abundance of preferred natural food resources that may be more profitable than supplementary food, either nutritionally or energetically, with many seed-eating bird species selecting diets to meet these requirements (Molokwu *et al.* 2011). Tree count data collected by the RSPB between 2013 and 2016 across woodlands within the Wye Valley and North Wales indicated that woodlands in the former were dominated by Common Ash, Common Beech, and Common Hazel *Corylus avellana*, while North Wales woodlands had high proportions of birch *Betula* spp., Common Ash and oaks (Stenhouse *et al.* 2023c). This difference in tree species composition may have resulted in Hawfinch populations in North Wales exploiting more readily available supplementary resources while reducing the searching time for preferred natural foods if they were scarcer in the environment (MacArthur & Pianka 1966, Stenhouse *et al.* 2023c). The significant difference between the presence of supplementary food between regions further supports these suppositions.

The lower prevalence of supplementary food within the diet of Hawfinches within the Wye Valley suggests that preferred natural food resources, such as Common Beech and oak, may have greater availability for Hawfinches (Stenhouse *et al.* 2023c). However, delineating between core and secondary diet within this study remains arbitrary. Future research to set objective criteria that differentiate between the two diets would be beneficial, possibly through the determination of the macronutrient content of dietary items detected in North Wales and Wye Valley Hawfinches (Cuff *et al.* 2021). Additionally, further research into supplementary food use in winter would be valuable, as other supplementary foods (e.g. peanuts) may be detected, giving an idea of the broader influence of supplementary food on Hawfinches.

The GLMM revealed a significant difference in sunflower prevalence between years. This difference may be due to natural temporal variations in food availability, as well as climatic differences impacting the birds' foraging ability. Resource pulse events, such as seed masting, can provide short-term increased resource availability for woodland birds (Nussbaumer *et al.* 2021). While it was not possible to explore whether we were sampling within a mast year during this study, it is plausible that Hawfinches would have been utilizing natural food resources at different frequencies between sampling years. Localized or short-term changes in temperature, rainfall and wind can also heavily influence individual behaviour and physiology, impacting birds at a population and species level (Wiley & Ridley 2016). Again, while climatic data were not available in the analysis, it is conceivable that climatic differences between sampling years would heavily influence Hawfinch foraging behaviour and, therefore, affect how frequently they utilized supplementary food resources.

Our results showed no sexual differences between the occurrence of supplementary food in the Hawfinches' diet. This was slightly surprising, as female birds are often less dominant than males and are usually outcompeted at feeders (Enoksson 1988, Hanmer *et al.* 2022). While biometric data were not included within this study, Hawfinches are considered to have minimal sexual dimorphism, which may mean there is less of a sex-based hierarchy. Personal observations at the feeding sites also showed that female and male birds that were obviously paired often fed alongside each other. It has also been noted that, especially during the winter months, UK Hawfinch populations are bolstered by over-wintering birds from continental Europe (Kirby *et al.* 2015). Based on this and the personal observations, it can be tentatively suggested that Hawfinches do not show intra-specific competition. During the breeding season, females and males are expected to have different energetic demands due to differential reproductive roles (da Silva *et al.* 2020). The similarity in supplementary food occurrence therefore suggests that foraging behaviours and the nutritional benefit of supplementary food are similar for both sexes.

An important caveat in our study was the use of sunflower seeds as a food source attractant at the artificial feeding sites. DNA metabarcoding was deemed a suitable method for detecting food items within the Hawfinch faecal samples, as it has been applied extensively to explore herbivorous elements of the Hawfinch diet within the UK and continental Europe (Stenhouse *et al.* 2023a, Stenhouse *et al.* 2023b). The percentage of Hawfinches with sunflower seeds in their diet is a good measure of a given population's overall dependence on this food, even though all birds must to some extent be using the artificial feeding sites.

The lower detection rate of sunflower may be linked to seed retention time within the Hawfinches' gut. Birds have been shown to have a relatively high efficiency when digesting carbohydrate-rich foods (McWhorter *et al.* 2009). It is therefore plausible that we detected supplementary food consumed by caught Hawfinches only a few hours earlier or during the previous day, before the birds had fed again in the morning. To assess if the use of sunflower seeds is a good measure of overall food availability or habitat quality, feeding trials and measuring relative read abundance (RRA) of sunflower seeds within the Hawfinch diet should be undertaken (Littleford-Colquhoun *et al.* 2022).

While our sample size of 286 was deemed adequate for the results and conclusions drawn from this study, it is important to consider that the results are based only on individuals who were captured and tested, which is only a small part of the population. Individuals caught during the study period may be bolder or have specific energetic or nutritional demands that resulted in them using the feeding sites to specifically feed on sunflower seeds. However, Hawfinches can be difficult to study without utilizing the fieldwork methods detailed in this study.

It is important to note that some study sites had a relatively small number of samples collected (north Cardiff $n = 7$, East Anglia $n = 7$). As such, inferences regarding the occurrence of supplementary feeding within these regions are more tentative. For future studies, increasing the number of samples collected across all regions would be beneficial.

Metabarcoding can provide highly resolved dietary information regarding the prevalence of food resources. To gain a better insight into supplementary food use by Hawfinches, this information could be combined with plant phenology data to highlight periods where natural food availability may be low. This could, in turn, inform when providing food might be most beneficial and avoid over-provision, which may lead to dependence and increased disease risk. The specific nutritional and energetic requirements of Hawfinches have not yet been explored, and little is known about how these are met by natural or supplementary foods. Further research in this area may inform which types of supplementary food are most suitable.

Conclusion

The results raised in this pilot study highlight the importance of monitoring the use of supplementary feeding, within the context of gardens and conservation management. The spatial difference in supplementary food consumption may be due to differences in interspecific competition or in forest management affecting tree species prevalence in the Hawfinch's primary foraging habitat. While the benefits of supplementary feeding have been well-documented for many bird species, possible negative impacts should be considered alongside appropriate mitigation strategies (Siriwardena *et al.* 2007, Plummer *et al.* 2019, Shutt & Lees 2021). This has implications for targeted feeding used within a conservation project setting, such as provisioning seed to farmland birds to augment depleted food resources (Siriwardena *et al.* 2007, Hanmer *et al.* 2022). Further research is needed into the finer ecological impacts of supplementary feeding in general, for example in summer versus winter, to enable the improvement of best-practice guidance (Murray *et al.* 2016, Lawson *et al.* 2018).

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No potential conflict of interest was reported by the author(s).

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Data availability statement

All data supporting the work presented in this study are openly available on Dryad at https://doi.org/10.5061/dryad.0p2ngf25d.

ORCID

Ewan H. Stenhouse http://orcid.org/0000-0002-2524-1914
Will B. Kirby http://orcid.org/0000-0002-1785-8252
William Bernard Perry http://orcid.org/0000-0001-9596-3333
Angela Marchbank http://orcid.org/0000-0002-6145-2436
Trudy Workman http://orcid.org/0000-0003-1687-1516
Paul Bellamy http://orcid.org/0000-0003-2234-2119
Ian P. Vaughan http://orcid.org/0000-0002-7263-3822
William O.C. Symondson http://orcid.org/0000-0002-3343-4679
Pablo Orozco-terWengel http://orcid.org/0000-0002-7951-4148

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