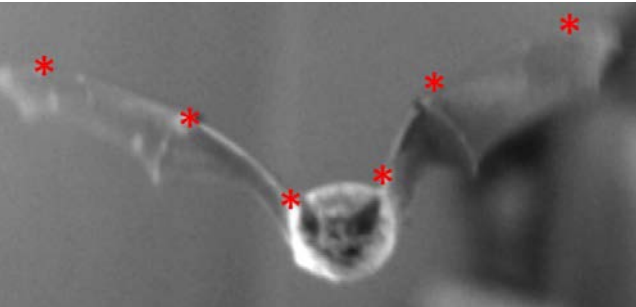




Measuring the effect of wing tears on flight in common pipistrelle bats (*Pipistrellus pipistrellus*)



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ABSTRACT

Bat wings are susceptible to tearing. Many bats are admitted to care with wing tears and their flight is subjectively measured prior to release. This study presents a new method to objectively measure the effect of bat wing tears on the flight of common pipistrelle bats, *Pipistrellus pipistrellus*. Bats were filmed and their wing movements and body positions tracked using freely available software. Results found that bats with bilateral tears moved their wings with smaller movements, and with more wing beats per second. Bats with wing tears tended to tilt their whole body towards the healthier wing - which is the wing with no or smaller wing tears. Differences in wing movements and body positioning suggest that flight might be affected in bats with wing tears, and future work should assess whether foraging and survival are also affected in these animals.

INTRODUCTION

All bat wing membranes are flexible; their musculature and skeletal supports enable the wings to be moved and their shape modified during flight (Skulborstad et al. 2015). These movements generate appropriate lift and thrust to allow the animal to be manoeuvrable during flight (Vaughan 1970; Swartz et al. 1996; Neuweiler 2000). Bat wing membranes are divided into sections by the skeletal fingers of the hand. These sections are likely to play different roles during flight; the proximal section (the plagiopatagium) provides lift and supports the body (Vaughan 1970; Swartz et al. 1996; Neuweiler 2000), while the distal sections (the chiropatagium) provide thrust (Swartz et al. 1996; Neuweiler 2000). Wing membranes are large to support flight; however, they are also thin and delicate, which makes them prone to tearing (Ceballos-Vasquez et al. 2015). Wing tears are thought to affect flight manoeuvrability and energetics (Voigt 2013), although some bats have been recorded flying with large wing tears, including the Pallid bat (*Antrozus pallidus*), Sliver-tipped Myotis (*Myotis albescens*) and Black Myotis (*M. nigricans*) (Davis 1968; Voigt 2013). Approximately 2000 bats with torn wings are taken to rescue centres for

rehabilitation annually in the UK (Hazel Ryan, Personal communication; Maggie Brown, Personal communication), which especially affects the most abundant *P. pipistrellus*. There is currently no clear recommendation for how soon rehabilitated bats can be released following a tear, and usually bat carers subjectively judge flight-tests by eye to decide when bats are ready (Khayat et al. 2019). Previous studies have developed complex experimental set-ups for measuring bat flight, including measuring 3D wing kinematics (Riskin et al. 2008; Schunk et al. 2017), wing vortices (Hedenström et al. 2007; Muijres et al. 2008; Hubel et al. 2016) and even electromyography (Konow et al. 2017). These set-ups can measure the precise biomechanics of bat flight; however, they are expensive and difficult for non-specialists to use. Indeed, studies of this nature are only useful for rehabilitation purposes if the methods are simple, or the results can be distilled down into key messages for the bat carer to use to judge healthy flight for release. This study, therefore, presents a simple method to objectively measure the effect of bat wing tears on the flight of *P. pipistrellus*.

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METHODS

Over a period of three summers, 36 *P. pipistrellus* bats were filmed flying at Lower Moss Wood Nature Reserve, Cheshire, and the Wildwood Trust, Kent, as well as at bat carers' houses in South Lancashire, Sussex and Yorkshire. All bats were wild but in care during this study. Bats were all kept individually in smaller cages, tanks or *flexaria*, and exercised in an indoor (Lower Moss Wood Nature Reserve) or outdoor flight cage (the Wildwood Trust, and some bat carers), or a spare room (bat carers), to prevent muscle atrophy. All bats were assessed before the study in accordance with the Bat Conservation Trust Bat Care Guidelines (Miller 2016); carers made sure each bat was a healthy weight, feeding, grooming, echolocating and stretching their wings normally. Some, but not all, bats had made test flight prior to filming. The filming, and subsequent analyses were only carried out on bats that could fly (36 bats), with partially healed injuries or small wing tears. Before filming, the bats were photographed with extended wings (Figure 1a) in order to record any tears. These were categorised as either holes (small puncture hole), contained tears (whole tear contained within the wing), total tears (complete tear on wing membrane to the trailing edge) or trailing edge tears (horizontal tear at trailing edge of wing), according to the scoring system developed in Khayat et al. (2019) (see example tear in Figure 1a). Tear position was recorded (Figure 2a and b) and the area measured to approximate the percentage size of the tear (*tear size (%)*) (Khayat et al. 2019). The difference in percentage tear size between each wing was also calculated to give *bilateral tear asymmetry (%)*. Each bat was also categorised as having no tears (n=10), unilateral tear(s) only on one wing (n=16), or bilateral tear(s) on both wings (n=10). The most injured wing was also identified in bats with bilateral tears, since one wing tended to have a much larger tear (with mean bilateral tear asymmetry values of 77.9%). Therefore, the healthier wing is defined as the wing without injury (in bats with tears on one wing) or the wing with the smallest tear (in bats with tears on both wings). Bats were warmed in a cloth bag or towel, until they were

active and warm. They were then placed on to a towel or gloved hand, which was held away from the carer at around head height, until the bat flew around the space where they were usually assessed for flight. This included a spare room at bat carers' houses (5 x 3 x 3 m), an indoor corridor (6 x 3 x 3 m) at Lower Moss Wood Nature Reserve, and outdoor flight cages (8 x 4 x 2.3 m) at the Wildwood Trust and one bat carer's house (Figure 1c). Flying was conducted during daylight hours, so the bats were clearly visible, and lasted until the bat tired and was returned to its cage. Filming was conducted using a high-speed camera (Phantom Camera, MIRO_M110, 200 fps, 1280 x 800 pixel resolution). The camera was manually triggered and 3-12 video clips lasting five seconds were captured from each bat. Each clip was reviewed and selected for analysis when the bat was: i) clearly in view, ii) directly front-on, or back-on to the camera (so both wings were clearly visible and looked symmetric) and iii) flying straight, so not tilting or turning (i.e. only 0-5° of body tilt), for one second. This gave a total of 276 clips for tracking.

Videos were tracked using the Manual Whisker Annotator (MWA) (Hewitt et al. 2016). Two points (black arrows in Figures 1b and d) were tracked on the left and right side of the body and used to measure *body orientation* from the horizontal (in degrees, θ_{BO} in Figures 1b and d); such that 0° body orientation was horizontal, negative body orientation was tilting right-wards, and positive body orientation was tilting left-wards (Figure 4a). Three points were tracked on the upper edge of each wing (red asterisks in Figures 1b and d). These points were used to calculate a wing angle perpendicular to the body (in degrees, θ_{WA} in Figures 1b and d); such that a 180° wing angle was straight up and 0° wing angle was straight down. The wing angles were then used to calculate *maximum wing angle* (degrees) (Figure 1d) and *minimum wing angle* (degrees) (Figure 1b). *Wingbeat frequency* (Hertz) was also calculated by counting the wing beat cycles visually in each clip. All wing measurements were calculated for both wings.

RESULTS

Tear Description

In bats with unilateral and bilateral wing tears, the tears present were mainly in the proximal wing section, the plagiopatagium (P), which is the closest section to the body (Figures 2a and b). Indeed, bats with unilateral wing tears had significantly more tears in the plagiopatagium (P) section than the chiropatagium (CI and CII) sections (Chi-squared Tests: $\chi^2 = 14.362$, $df = 2$, $p = 0.001$). However, there was no significant difference in tear number between the wing sections in bats with bilateral wing tears (Chi-squared Tests: $\chi^2 = 1.750$, $df = 2$, $p = 0.417$). The number of tears did not significantly differ between bats with unilateral and bilateral wing tears (Chi-squared Tests: $\chi^2 = 0.581$, $df = 1$, $p = 0.446$) (Figures 2c, 2d). The most common type of tear was 'hole' (Figures 2c, and d), although numbers were not significantly different from the other tear types (Chi-squared Tests: $\chi^2 = 7.231$, $df = 3$, $p = 0.065$). Tear type did not significantly

vary between bats with unilateral and bilateral tears (all Chi-squared Tests: $p > 0.05$).

Wing measurements

A Wilcoxon test showed that there were no significant differences between the left- and right-wing movements (all p 's > 0.05) (Table 1). Therefore, for graphing (Figure 3), data from the two sides were combined for the bats with no tears and bilateral tears and kept separate for those with unilateral tears. Bats with bilateral tears had significantly lower maximum wing angles (Kruskal-Wallis Test: $\chi^2 = 16.287$, $df = 2$, $p < 0.001$), higher minimum wing angles (Kruskal-Wallis Test: $\chi^2 = 14.732$, $df = 2$, $p = 0.001$) and higher wingbeat frequencies (Kruskal-Wallis Test: $\chi^2 = 10.580$, $df = 2$, $p = 0.005$) than bats with no tears or unilateral tears (Figure 3). There were no significant correlations (Spearman's Rank correlation: $p > 0.05$) between any wing measurement and tear size or bilateral tear asymmetry.

Body orientation

Bats with no wing tears had a horizontal body orientation (mean θ_{BO} $0.03^\circ \pm$ s.d 14.49°), whereas bats with unilateral or bilateral wing tears had larger deviations in body orientations (Figure 4b; Kruskal-Wallis Test: $\chi^2 = 6.111$, $df = 2$, $p = 0.047$). The bat's body generally oriented towards the most intact wing, both when the largest tear was on the left wing (unilateral wing tears: $-1.08^\circ \pm 14.31^\circ$, Mann-Whitney U Test: $Z = 1.515$, $p = 0.014$; bilateral wing tears: mean = $-5.12^\circ \pm 12.84^\circ$, Mann-

Whitney U Test: $Z = 780$, $p = 0.006$) (Figure 4, left panels, in white), and also the right wing (unilateral wing tears: mean = $4.67^\circ \pm 12.37^\circ$, Mann-Whitney U Test: $Z = 1.515$, $p = 0.014$; bilateral wing tears: $3.96^\circ \pm 9.86^\circ$, Mann-Whitney U Test: $Z = 780$, $p = 0.006$) (Figure 4, right panel, in black). Despite body orientation being affected by wing tears, there were no significant correlations (Spearman's Rank correlation: $p > 0.05$) between body orientation and tear size or bilateral tear asymmetry.

Figure 1: Filming bats with wing tears. a) photograph of a torn wing, showing a total tear; b) example video still of body orientation (θ_{BO} in black) calculated from the horizontal line from the two body points (black arrows); and minimum wing angle, showing wing angles (θ_{WA} in red) calculated from the red dotted line, perpendicular to the body orientation, to the most distal tracked point on the wing (*); c) the filming set-up of high-speed video camera filming in a corridor; d) example video still of maximum wing angle, using the same definitions as in panel b.

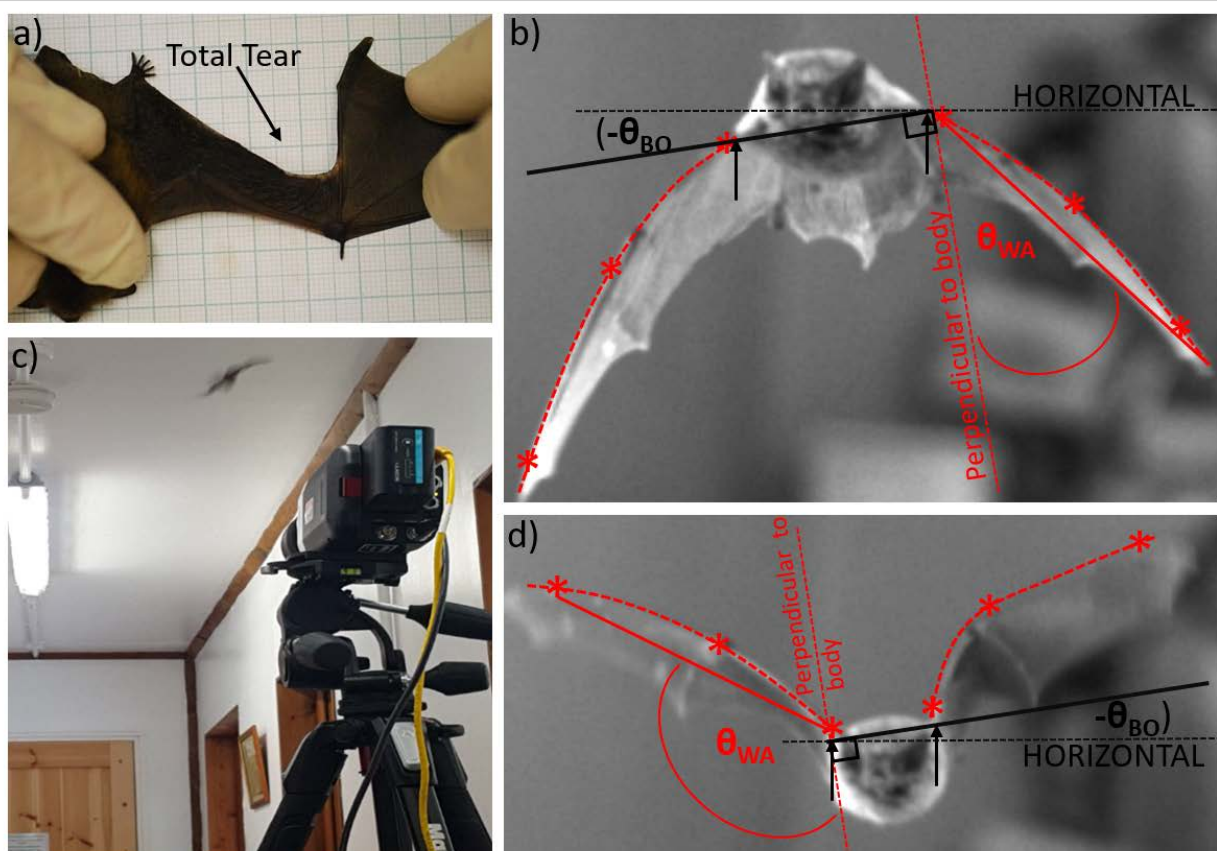


Figure 2: Description of wing tears. Panels a) and b) show the distribution of different types of tears over the wing sections for bats with unilateral and bilateral tears. Panels c) and d) present the total count of each tear type in each section of the wing: the distal chiropatagium section (CI), the proximal chiropatagium section (CII) and the plagiopatagium section (P).

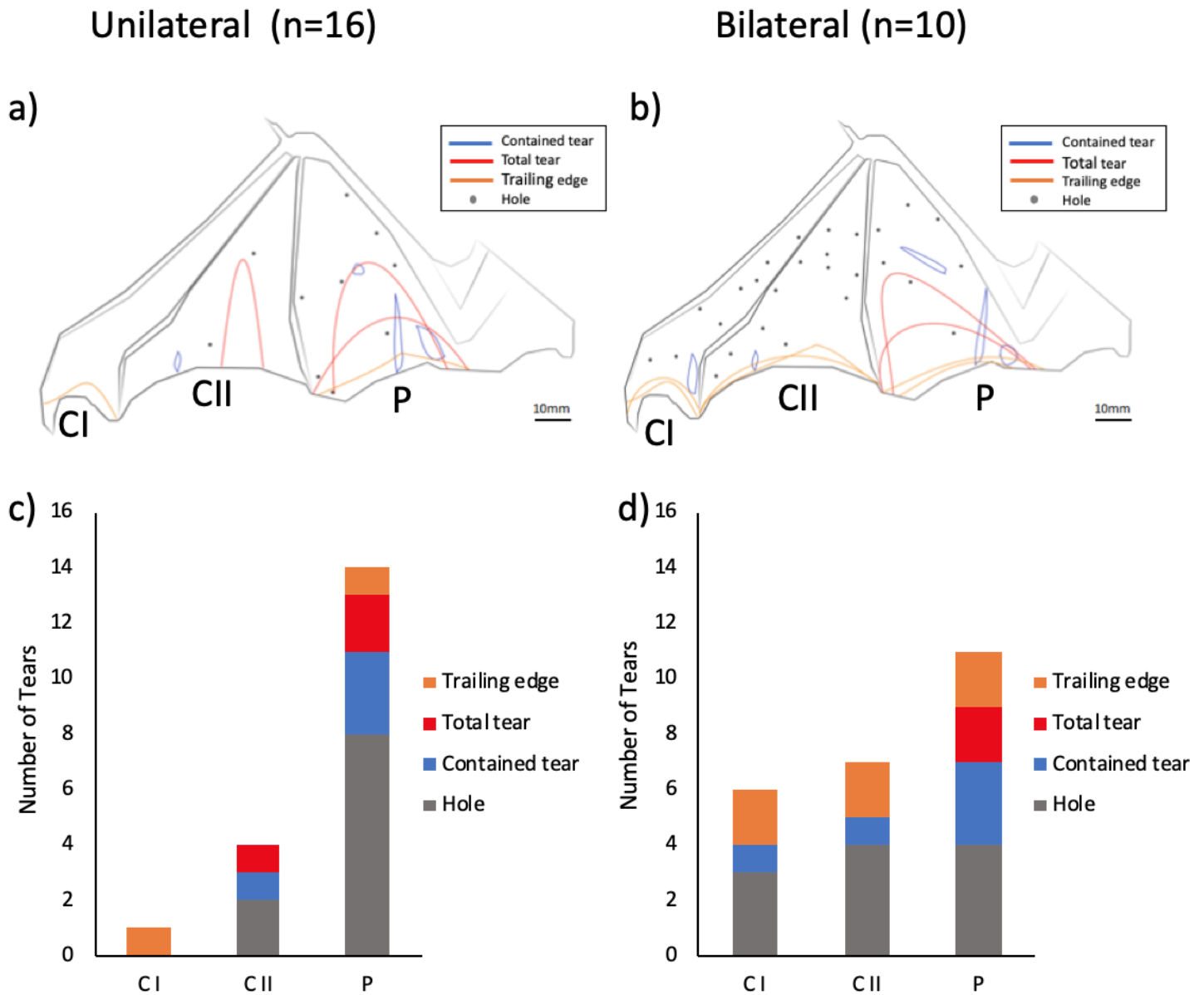


Table 1: The Wilcoxon tests to compare the left and right wing in the in bats with no tears, bats with unilateral wing tears and bats with bilateral wing tears. Data is from 10 animals for no tears, 16 animals for unilateral wing tears and 10 animals for bilateral wing tears.

	No tears	Unilateral	Bilateral
Maximum angle	Z=-0.018, p=0.985	Z=-0.819, p=0.413	Z=-0.808, p=0.419
Minimum angle	Z=-0.135, p=0.892	Z=-0.434, p=0.665	Z=-0.104, p=0.917
Mean angle	Z=-0.263, p=0.792	Z=-1.188, p=0.235	Z=-0.450, p=0.653
Amplitude	Z=-0.691, p=0.490	Z=-0.387, p=0.699	Z=-1.444, p=0.149
Wing Frequency	Z=0.000, p=1.000	Z=-1.802, p=0.072	Z=0.000, p=1.000

Figure 3: Wing measurements from the video analysis: a) Maximum wing angle; b) Minimum wing angle; c) Wingbeat frequency, in bats with no tears, unilateral wing tears and bilateral wing tears. Bats with bilateral wing tears have significantly different wing measurements compared to bats with no or unilateral wing tears. Graphs show mean values with standard error bars (* $p < 0.05$).

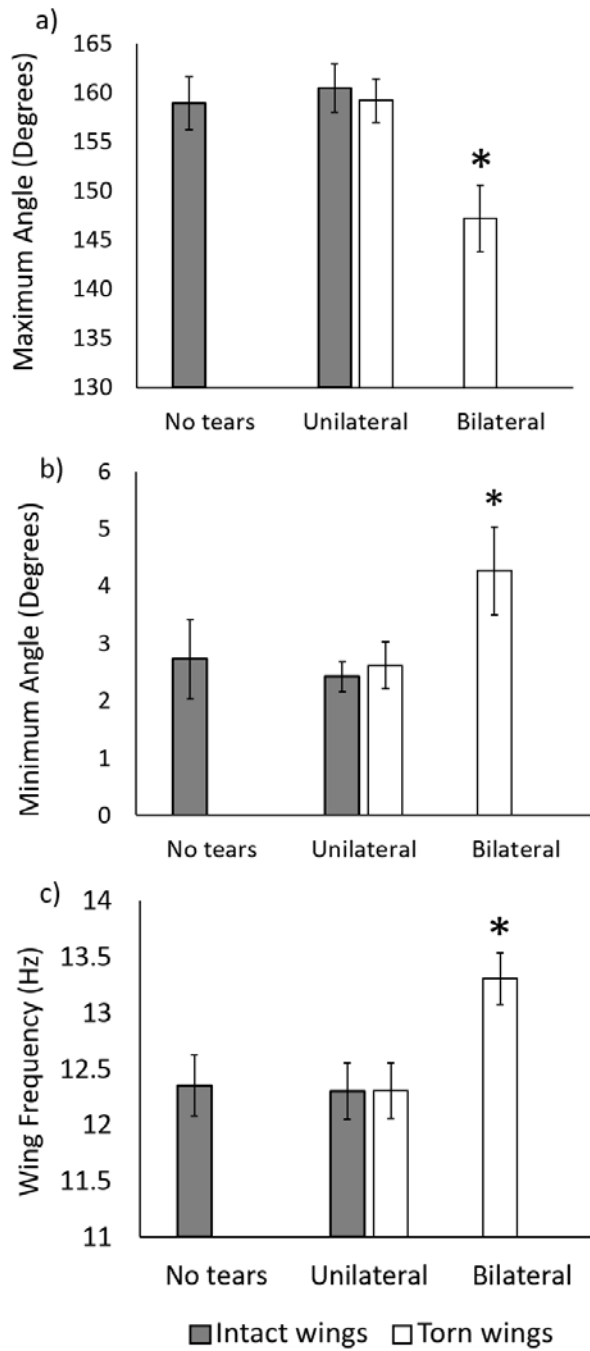
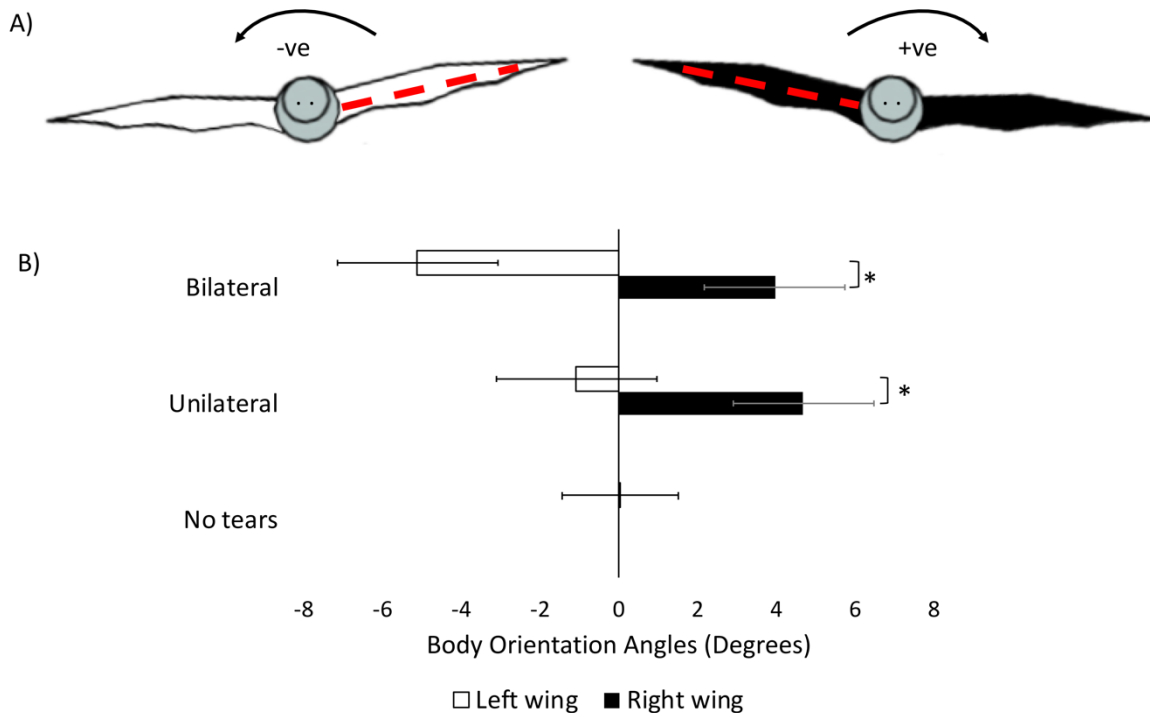


Figure 4: Body orientation measurements from the video footage. a) Diagram presents how the body leans towards the most intact wing (red dashed line); i.e. the bat leans toward the right (negative body orientation) when the tear/ largest tear is in the left wing (white colour), or leans toward the left (black colour) (positive body orientation) when the tear/ largest tear is in the right wing. b) Body orientation in bats with no tears, unilateral wing tears, and bilateral wing tears, when the tear/ largest tear was found in the left (white) or right (black) wing. Graphs show mean values with standard error bars (* $p < 0.05$).



DISCUSSION

Results showed that bats with bilateral wing tears had overall reductions in wing movements with lower maximum wing angles and higher minimum wing angles (Figures 3a and 3b). Their wings also moved with a higher frequency ($>13\text{Hz}$) (Figure 3c); possibly to compensate for the smaller wing movements and ensure appropriate lift and thrust for flight (Norberg & Norberg 2012). There is a complex interplay between wing amplitude and wing beat frequency, which affects aerodynamics and flight energy expenditure (Taylor et al. 2003). Any change from a preferential movement frequency is likely to be less efficient (Taylor et al. 2003; Norberg & Norberg 2012); therefore, bats with bilateral wing tears are likely to have reduced flight efficiency. Indeed, wingbeat frequencies can also increase in birds with reduced wing areas and is also thought to increase flight costs (Hambly et al. 2004).

Bats with wing tears (unilateral and bilateral) also had a less horizontal body position (Figure 4b); they tended to lean towards the healthier wing, which might serve to take some of the weight or pressure off the more injured wing. Similar results have been found in quadrupedal mammals, such as in horses (Duberstein 2012) and rats (Ängeby Möller et al. 2012), where the animal will shift body weight off an injured limb towards the healthy side. Birds with asymmetric flight feathers have reduced manoeuvrability (Swaddle et al. 1996) due to differential lift on each wing (Thomas 1993).

All sections of the wing play key roles during flight (Vaughan 1970; Swartz et al. 1996; Neuweiler 2000).

Therefore, damage to different sections of the wing might affect flight in various ways. There were more tears in the proximal wing sections of the bats in this study, especially in the P section in bats with unilateral tears (Figure 2). However, all the 36 individuals could fly to some extent, and there was no clear association between tear size and placement on any of the wing or body orientation measures. Some of the bats had other injuries, such as to the joints, as well as internal injuries; these may contribute to some of the variation in flight that was observed here. While bat carers do log the incidence of injuries (as Miller 2016 recommends), it is not possible to judge internal injuries. In addition, the level of detail in the notes can vary between carers; therefore, we could not use these case notes consistently for further analyses. In future, reliably reporting all injuries would usefully compliment flight assessments as these injuries are also likely to impact flight. In addition, some of the bats had healed holes or tears; these would not be scored as current tears but may still impact flight as healed skin has a different pigment, reduced elasticity and lacks sensory hairs (Sterbing-D'Angelo et al. 2011).

Recommendations

We have developed a novel filming and tracking method to objectively assess bat wing movements and body orientation during rehabilitating flights. Results suggest that wing movements are affected in bats with bilateral wing tears, and body orientation is affected in all bats with wing tears. We suggest that counting the number of wing beats and looking at the orientation of the body during

straight, flat flight might be a good place to start assessing bat flight for release. In particular, wing beats of over 13 Hz (Figure 3c) and body orientations of more than four degrees from horizontal (Figure 4b) might suggest flight being affected in adult *P. pipistrellus* (note that juveniles have faster wingbeat frequencies). We recommend that filming with a slow-motion camera at 100-200 fps (such as a GoPro or iPhone camera), and making wing beat counts, or simple body or wing angle measurements using free software (such as MWA (Hewitt et al. 2016), Tracker (Brown & Wolfgang 2013) or ImageJ (Schneider et al. 2012)) is a useful way to start objectively monitoring bat flight. It might be possible to assess body orientation by eye, without cameras and tracking, to see if a bat is leaning extremely towards their healthy wing. In support of our body orientation results, we recently observed a bat with a unilateral tear on the left wing

leaning so much towards the intact right wing that it could only make right-hand turns. This suggests that turning and manoeuvrability could be even more affected by tears than the straight flight investigated here (in agreement with Voigt 2013; Pollock et al. 2016). In order to truly assess the effect of tears and their healing on flight, wing and body measurements should be taken throughout rehabilitation, as soon as the animals is able to fly, until just before release. Furthermore, if wing tears do significantly impact the ability of bats to forage successfully and survive, it is imperative to validate these wing and body measurements against survival outcomes in order to rehabilitate bats successfully. Indeed, objective guidelines for bat release need to be developed so carers can appropriately judge which injuries are serious for bats, and when they are ready for release.

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