Fog and dense gas disrupt mosquito flight due to increased aerodynamic drag on halteres

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ABSTRACT

Chemical insecticide foggers have long been used to control mosquito populations and reduce the spread of malaria, West Nile virus, and dengue fever. In this study, we show that simple fogs of water droplets, devoid of chemicals, are effective at obstructing the flight of *Anopheles freeborni* mosquitoes. Using high-speed video, we film the flight of mosquitoes in denser-than-air fluids such as fogs produced by household humidifiers and heavy gas. In gases with more than twice the density of air, mosquitoes cannot maintain flight stability, but pitch and roll uncontrollably until they fall to the ground. We here show that the increased drag of denser-than-air flight environments is sufficient to render a mosquito's gyroscopic sensors and wingbeat controllers, their halteres, ineffective. Anomalous drag forces are estimated with steady and unsteady drag analyses. We glue masses of 10 ng to each haltere, equivalent to 0.0005% the mass of the mosquito. Mosquitoes with such a minor treatment executed flight patterns, similar to mosquitoes in fog, indicating a mosquito's haltere are highly tuned to aerodynamic properties of normal air, making them sensitive to contaminants. The failure of mosquito flight in fog reveals potential new methods for mosquito control that do not require insecticide-laced particles.

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1. Introduction

Half of the world's population resides in regions where malaria transmission is a risk (World Health Organization, 2011). In 2010, approximately 216 million clinical malaria episodes resulted in an estimated 655,000 deaths. Transmission of malaria to humans occurs through the bite of an infected female mosquito (Becker et al., 2010). Mosquito control methods include intermittent preventative treatment such as removal of stagnant water pools (Focks et al., 2000) and usage of insecticide-treated bed nets (Lengeler, 2004). Indoor residual spraying (Pates and Curtis, 2005), chemical larviciding (Borovsky, 2003), and insecticide spraying (Britch et al., 2010) aim to kill adult mosquitoes. The latter technique works by depositing toxins on the insect body, through encounters with airborne particles or insecticide-ridden surfaces. Toxins are carried by water or oil micro-droplets. While these insecticides possess acceptably low toxicity to humans, strict regulations govern their use and application. Spraying and fogging should be done at the peak of adult mosquito activity, and requires repetition, which can be expensive. By studying the vulnerabilities of mosquito flight, unforeseen strategies for mosquito

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mitigation free of insecticides may be found. The chosen model organism in the study is the *Anopheles freeborni* mosquito, an established malaria vector.

In this study, we investigate mosquito flight vulnerabilities by probing the limits of their locomotion through particulate environments. Many insects, including mosquitoes, thrive in wet, humid locations such as the tropics (Khasnis and Nettleman, 2005). Abundant moisture often brings about heavy rains and dense fogs, through which mosquitoes must fly in search of food and mates. Introducing particles such as raindrops into the flight medium increases the cost of flight performance for bats and birds (Voigt et al., 2011). Previous work has shown that mosquitoes can survive rain showers (Dickerson et al., 2012) by virtue of their low mass which greatly reduces the force of impact. A mosquito's flight performance through dense fog is poorly understood, despite the widespread use of insecticide foggers. Little is known of how small particles can affect the insect's air–wing interactions and sensing of its environments.

Airborne particles can broadly be characterized into one of the two categories, fog and haze. Fog is defined as providing less than 1 km of visibility (Pinnick et al., 1978). Depending on the altitude (Gultepe et al., 2009), fog naturally consists of small water droplets, in the liquid or frozen state, of size 1–10 µm in diameter and concentrations 25–100 mg/m^3. Fog has a bimodal distribution; the larger fog particles close to the ground have an average diameter of 5 µm, whereas the higher altitude particles have a diameter of 0.3–0.6 µm. On the other hand, haze consists of 0.2 µm dust and smog particles, with little vertical variation. Fog and haze particle size can be measured much like insecticide particle size, through light scattering and holographic techniques (Sinclair and La Mer, 1949; Thompson et al., 1967). In comparison to natural fogs, insecticides involve drop sizes comparable to that of natural fogs, but of one-millionth the density, less than 100 ng/m^3 when dispersed, rendering them invisible (Seiber et al., 1993). However, during dispersion, fogs and sprays at output nozzles are very dense and visible.

Dense fog has been known to disrupt avian vision and navigation ability (Bevanger, 1994). Like birds, insects also use a combination of visual and force-mediated feedback to navigate (Bender and Dickinson, 2006). Tethered fruitflies and houseflies use an optomotor response to adjust their thrust and torque for visual cues positioned in front of their eyes (Götz, 1968). Insects without gyroscopes, like the desert locust, employ other means of flight sensing (Cheng et al., 2011; Arbas, 1986) such as sensitive hairs on their hind limbs to detect wind direction. The small tortoiseshell butterfly uses its antenna to detect flight speed (Niehaus, 1981; Gewecke and Niehaus, 1981), while the hawkmoth vibrates its large antennae to detect Coriolis forces (Sane et al., 2007). Only recently has any work been done on how particles affect olfactory sensors on cockroaches (Böröczky et al., 2013), but it is well-established that mosquitoes rely heavily on olfactory cues to find food and mates (Sutcliffe, 1994). However, the effect of airborne particles on these sensing systems has not been systematically studied, and sensing systems are very species specific.

In this study, we focus on flight sensing due to the interaction between fog and a mosquito's halteres. Halteres, small knobbed structures evolved from hind wings, flap with the same frequency as wings and serve several in-flight functions (Fraenkel and Pringle, 1938; Wu et al., 2002; Dickinson, 2003) (Fig. 1(a) and (b)). Halteres provide proprioceptive feedback by detecting Coriolis forces and play a crucial role in the neuromuscular circuit generating the wingbeat rhythm in Dipterans (Bartussek et al., 2013; Bender and Dickinson, 2006). By activating the motor neurons of minuscule steering muscles, halteres help regulate wing motion through a reflexive feedback loop with these muscles. Haltere-bearing insects have irregular fields of campaniform sensilla embedded in their cuticle at the base of the haltere shaft (Sane and McHenry, 2009). The architecture of the campaniform structures gives them the ability to detect small strains in the cuticle as the haltere flaps. Historically, halteres have been thought to sense inertial, centrifugal, and gravitational forces in addition to Coriolis forces (Wu et al., 2002).

Mosquito halteres have not been the focus of much investigation, but the function and dynamics of fruitfly halteres has been well studied. In tethered fruitflies, adding mass to one haltere using glue acts to reduce peak velocity and amplitude of body saccades (Bender and Dickinson, 2006). Ablating one fruitfly haltere has the opposite effect by accelerating in-flight maneuvers. In some situations, haltere augmentation results in the adoption of a flapping frequency different from that of the wings (Sellke et al., 1936; Pringle, 1948). Sensory feedback from the haltere is read by the wing control mechanisms on a stroke-by-stroke basis, even in steady flight (Heide, 1983; Fayyazuddin and Dickinson, 1999), so the effects of haltere augmentation in fruitflies may be due to the disruption in synchronous wing-haltere signals (Bender and Dickinson, 2006). Houseflies with halteres removed show a complete lack of control over flight (Fraenkel and Pringle, 1938).

In this study we investigate the ability of *A. freeborni* mosquitoes to maintain flight in hyperdense air. In Section 2, we present our measurements of the likelihood and promptness of flight failure in heavy air, and a calculation of the aerodynamic forces involved. To show that such forces are sufficient to perturb halteres, we laden halteres with additional mass. In Section 3, we present a discussion of our results and avenues for future work. Our experimental methods for insect handling and imaging are provided in Section 4, and we summarize our main points in Section 5.

2. Results

2.1. Flight failure in fog and heavy gas

We performed hundreds of experiments with *A. freeborni* flying in both fog and 38 mixtures of gas with various densities. Fog here refers to the output from a commercial humidifier, consisting of 5–35 µm diameter water droplets, with an average diameter of 15 µm. The microscopic droplets increase the aggregate fluid density through which a mosquito flaps. We define flight failure as a rapid descent to the ground and the subsequent inability to resume flight. Failure is observed when a mosquito encounters a stream laden with fog particles or when introduced to a quiescent medium with suspended fog
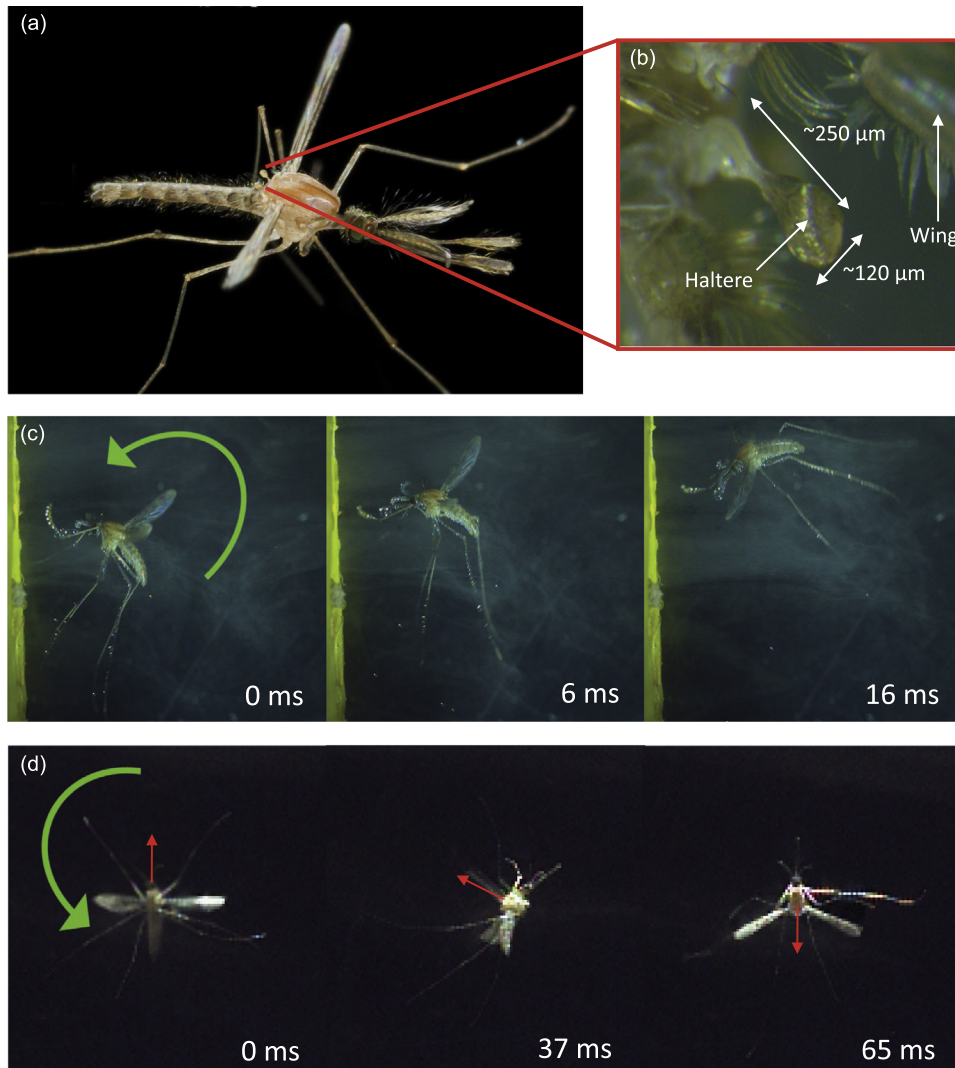
Fig. 1. (a) An *Anopheles freeborni* mosquito in flight. The location of mosquito's haltere boxed in red. (b) A mosquito haltere. A video sequence of a mosquito pitching forward (c) and rolling (d) while aloft in a stream of fog particles. The red arrows denote the direction normal to the mosquito's back. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

particles. Landing on the floor of a container is atypical behavior, as mosquitoes prefer to land on walls and ceilings, regardless of the species (Paaijmans and Thomas, 2011). We observe less than 10% of individuals from a group (number of trials, $N = 20$) of *A. freeborni* choose to rest on the floor of their container under normal conditions.

Every case of flight failure ($N > 100$) involves pitching (Fig. 1(c), Supp. Video 1) or rolling (Fig. 1(d), Supp. Video 2), generating inversion or steep angles of orientation with respect to the horizontal flight position. When mosquitoes have rotated 90° relative to vertical, we surmise that the lift generated by wings cannot maintain their position and they quickly begin falling downward. In other instances, the mosquito maintains its typical orientation and even generates lift in fog, or heavy gas, for fractions of a second, as shown by the upward flight in Supp. Videos 1 and 2. Nevertheless, these periods of normal flight, which occur in approximately 10% of the flights filmed, are so sporadic and unstable that mosquitoes inevitably fall to the floor within seconds of introduction of heavy fog. Fog particle deposition on the wings or body is not the cause of flight instability, as we observe that flight may resume immediately after fog particles are removed from the air, and that a mosquito blanketed with small drops on its wings and legs retains the ability to fly in dry air.

Mosquitoes behave similarly in both fog and heavy gas, indicating that the increased aggregate density of the medium is the cause of flight failure, rather than the particulate nature of fog. A heavy gas with the same average density as fog causes nearly identical flight failure, as shown in Supp. Video 3. Heavy gas is also transparent, indicating that the opacity of the medium and occlusion of vision is not a factor in their flight failure.

In Fig. 2(a) and (b) we present flight tracks for mosquitoes in normal air and hyperdense air, respectively. As demonstrated by the flight track in Fig. 2(a), a mosquito in normal air can remain aloft for more than 4 s before landing. Conversely, in hyperdense air, the descent behavior is clearly distinct. Fig. 2(b) shows the mosquito falls quickly from the
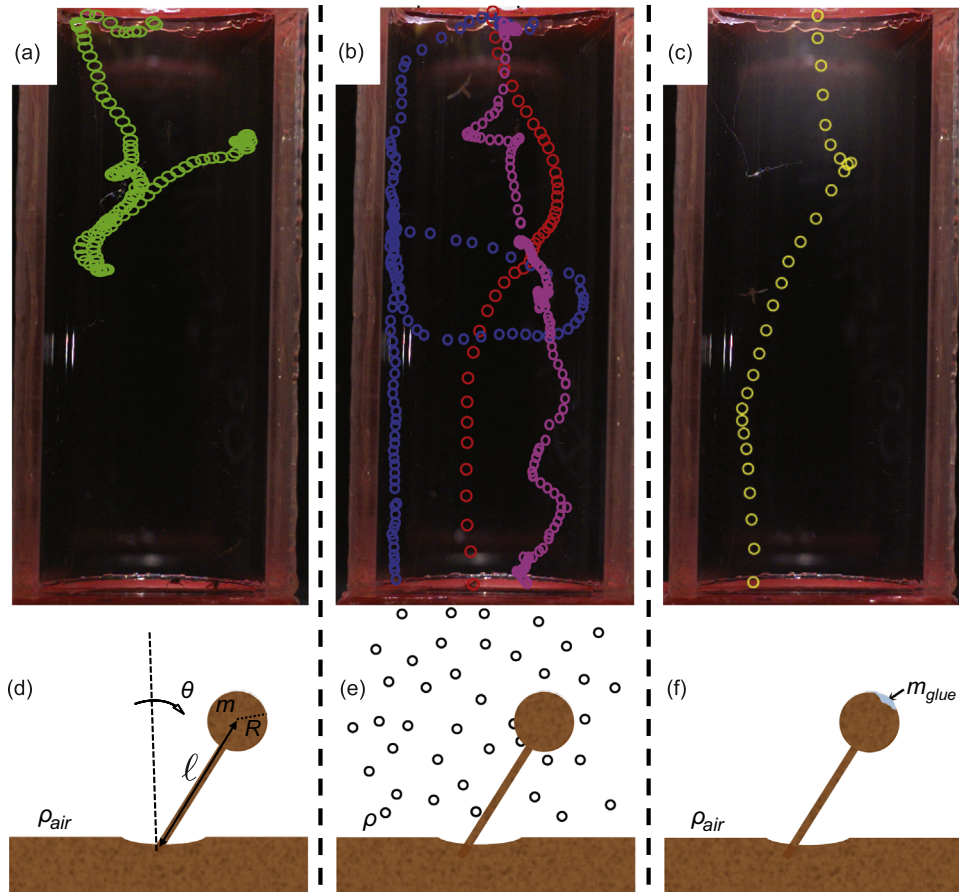
Fig. 2. Three columns designate flight in normal air, fog or dense gas, and flight with glue on halteres. Flight tracks (a–c) for a mosquito flying in normal air (a); in gas of density $\rho = 1.75$ (blue), 1.9 (pink), 2 (red) kg/m^3 (b); with glue deposited on its halteres (c). Schematics (d–f) of haltere flapping in normal air (d), in fog or heavy gas (e), with glue deposited on the tip (f). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

ceiling to the floor of the container. The mosquito falling in the most dense gas (red) shows the least control over flight, falling at an average falling speed of 27 cm/s over 0.55 s.

We measured the flight time before grounding when the mosquito is dropped from a height $H = 15$ cm in air made heavier by the addition of R134a. Humidifier fog was not employed in this experiment due to its propensity for settling and aggregating on container walls. Fig. 3(a) shows that descent time becomes both shorter and more predictable with increasing gas density. The fastest possible descent time is $T = 0.11$ s, the time needed to fall a distance H at terminal velocity, 136.5 ± 9.5 cm/s ($N = 10$). The shortest descent times observed are twice this minimal time, indicating mosquitoes are slowing their descent by attempting flight. During descent, mosquitoes flap their wings at varying frequencies (150–400 Hz), but continue to fall on their sides or inverted.

Fig. 3(b) shows the relation between gas density and proportion of mosquitoes able to sustain flight for over 3 s. Over an intermediate range of gas density, 0.9–1.6 kg/m^3, mosquitoes can stay aloft indefinitely. The corresponding success rate in this range is slightly less than 100% because of experimental error. In our experiments, we do not control for initial orientation of the mosquito. Thus, a few mosquitoes are released upside-down upon entry into the container and, if unable to recover in less than one second, strike the ground.

At higher gas densities, between 1.6 and 2 kg/m^3, only 50% mosquitoes can maintain steady flight. At the highest gas densities tested, greater than 2 kg/m^3, no mosquitoes are able to maintain flight. At this threshold, the volume fraction of water in the flight medium is 8×10^{-4}, and a cubic millimeter contains approximately 450 droplets. To investigate further the mechanism for flight failure, we consider in the next section the aerodynamic forces on the mosquitoes' flapping halteres.

2.2. Flight failure due to haltere–fluid interaction

In our previous section, we identified the air densities in which mosquitoes can fly. Mosquitoes fail to sustain flight in humidifier fog, which has a density up to 2 kg/m^3. We hypothesize that this inability to fly is caused by the interaction between the mosquito haltere and ambient fluid. Furthermore, after landing, mosquitoes remain grounded until the fog
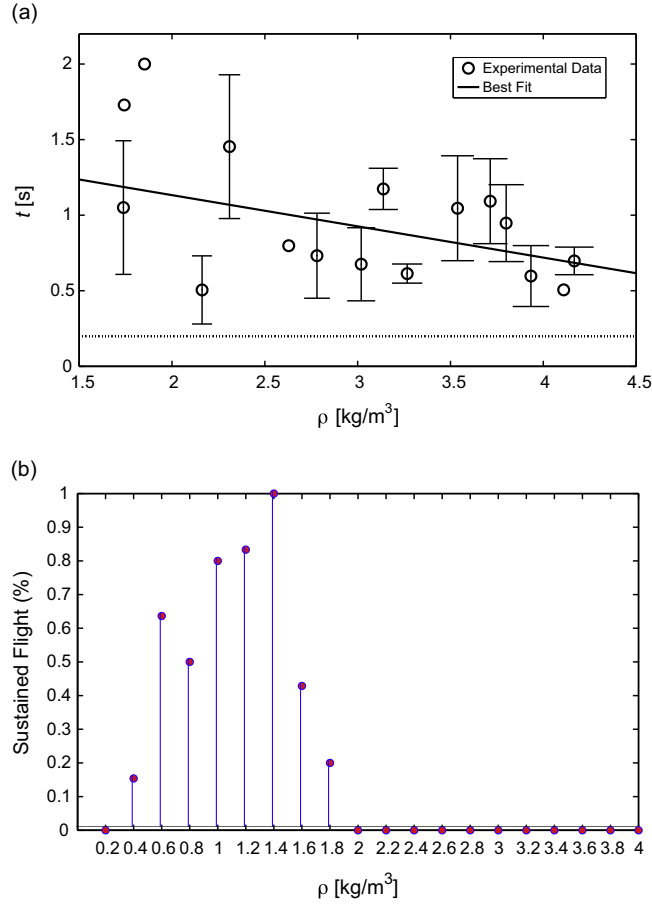
(a)

(b)

Fig. 3. (a) Relation between gas density ρ and descent time for mosquitoes suddenly introduced into a container of dense gas. Error bars denote $\pm$ one standard error. Trials were performed once per animal. Points without error bars denote a single trial at the specified gas density. The dotted line at the bottom bounds the minimum time required to fall a distance equal to the height of the container at terminal velocity. (b) Relation between gas density ρ and percentage of mosquitoes able to sustain flight. Each bar represents $N = 4$–12 trials.

particle concentration falls below 1.7 kg/m^3. Thus, it appears that mosquitoes can also sense fog in their surroundings and consequently choose not to take off.

We measure haltere kinematics and geometry using high speed videography and scanning electron microscopy. Halteres are drumstick-shaped, with a characteristic length of $\ell = 250$ μm and radius, at the widest point, of $R = 60$ μm, as shown in Fig. 1(a) and (b). In comparison to fog droplet of diameter 5–35 μm, a haltere has 5–1730 the volume. When tethered mosquitoes are stimulated, they flap both wings and halteres for brief periods using a full range of motion. We observe that haltere flapping is sinusoidal with similar kinematics for all gas densities tested (0.2–4.2 kg/m^3). In normal air, wings and halteres flap at a frequency of $f = 375 \pm 42$ Hz ($N = 19$) on the same order as the flapping frequency in dense fog ($\rho = 2$ kg/m^3) of $f = 303 \pm 37$ Hz ($N = 9$) (P-value < 0.0001). The 20% decrease in flapping frequency due to a 67% increase in medium density suggests that form drag on the wings is constant within both fog and air. Drag force $F_D \sim \rho f^2$ and $1.67 \times 0.8^2 \approx 1$.

In the following calculations, we use a characteristic flapping frequency of $f = 300$ Hz. The haltere flapping amplitude is $A = 97 \pm 2°$ ($N = 6$). The time-dependent, linear velocity of a haltere in simple harmonic motion can be written as $V(t) = A\ell\omega \cos(\omega t)$, where $\omega = 2\pi f$. The peak velocity of the haltere-tip is therefore $V_{max} \approx 2\pi f A\ell = 0.74$ m/s, using flapping frequency of $f = 300$ Hz, and flapping amplitude $A = \pi/2$ radians. At densities where flight failure begins, more than 300 fog particles are contained within 1 mm^3 of a mosquito's flight medium. The flapping halteres will encounter upwards of 2000 of these particles in 1 s of flight, assuming that the shaft's frontal area is negligible compared with that of the bulb.

The total force on the flapping haltere in hovering, non-rotational flight consists of a sum of drag force $F_{D,s}$, and inertial force F_i,

$$F_{haltere} = F_{D,s} + F_i, \tag{1}$$

where each term may be expressed as

$$F_{D,s} = \frac{1}{2}C_D\rho\pi R^2 V^2, \tag{2}$$

$$F_i = m\frac{dV}{dt}. \tag{3}$$

The haltere has mass m, radius R and time-dependent velocity V. Dimensionless coefficient $C_D = 5.1$, the drag coefficient for a sphere in steady flow. The corresponding Reynolds number Re $= 2R\rho V_{max}/\mu = 8$, for a medium density of 1.7 kg/m^3, and dynamic viscosity of air $\mu = 1.98 \times 10^{-5}$ kg/m s.

For flight medium density $\rho = 2.0$ kg/m^3 about 67 % greater than that of air ($\rho_{air} = 1.2$ kg/m^3), the anomalous peak drag force is $\Delta F_{D,s} = \frac{1}{2}(\rho - \rho_{air})C_D\pi R^2(A\ell\omega)^2 = 12.4$ nN, which is applicable only in the case when wings are not pushing air over the halteres. We believe this to be true of hovering mosquitoes through observations of haltere path and wing form.

The flapping amplitude of a flapping haltere is not small, and as such falls outside the governing assumptions of most theoretical studies of drag on a spheres in unsteady motion (Karanfilian and Kotas, 1978; Mei, 1994). However, we may approximate the magnitude of form drag and virtual mass forces of a sphere in unsteady motion for comparison to the value given by Eq. (2). Boussinesq and Basset show that unsteady drag force for spheres in unsteady motion the Navier–Stokes equations give (Boussinesq, 1885; Basset, 1888).

$$F_{D,u} = 6\pi\rho\nu RV + \frac{1}{2}m_{gas}\frac{dV}{dt} + 6\rho(\pi\nu)^{1/2}R^2\int_0^t \frac{f(t-\tau)}{\tau^{1/2}}\,d\tau, \tag{4}$$

where kinematic viscosity $\nu = \mu/\rho$, and $f(t-\tau) = dV/dt$, and m_{gas} is the mass of gas occupying the same volume as a haltere modeled as a sphere (Karanfilian and Kotas, 1978). The first term is form drag due to steady motion, the second is the virtual mass force arising from unsteady motion, and the third is drag from history of motion. Eq. (4) reduces to the drag equation given by Stokes (1851) for simple harmonic motion (Stokes, 1851):

$$F_{D,u} = 6\pi\nu\rho R\left[1 + \left(\omega R^2/2\nu\right)^{1/2}\right]V + \frac{1}{2}m_{gas}\left[1 + \frac{9}{2}\left(2\nu/\omega R^2\right)^{1/2}\right]\frac{dV}{dt}. \tag{5}$$

As before, we calculate the change in peak force on the flapping halteres by increasing medium density $\rho = 2.0$ kg/m^3 from that of standard air. The anomalous peak force of a sphere in simple harmonic motion $\Delta F_{D,u} = 17.0$ nN, very close to the estimate $\Delta F_{D,s}$ achieved by steady drag analysis. The values of $\Delta F_{D,s}$ and $\Delta F_{D,u}$ are 0.06% and 0.08% the weight of a mosquito, respectively, nearly equal to the haltere weight, estimated by volume to be 900 ng using the density of water (Nalbach and Hengstenberg, 1994).

Based on our observations of flight failure, we hypothesize that the calculated anomalous aerodynamic force of 12.4–17.0 nN in the high density medium is sufficient to incapacitate the haltere's function. In terms of haltere anatomy, such aerodynamic forces translate to increased strain detected by the campaniform sensilla embedded at the haltere shaft's base. A cantilever beam' strain $\epsilon \sim P$, where P is a point force at the tip. Therefore, the increased drag force corresponds to a 67% increase in strain registered at the base. In this case, the finely tuned haltere becomes slightly out-of-sync with the wing's steering muscles, which in turn creates the rapid pitching and rolling behaviors observed in flight.

2.3. Flight failure due to artificially increasing haltere mass

To test our hypothesis that aerodynamic forces of 12.4–17.0 nN incapacitate halteres, we perform experiments with mass added to both halteres. Mass added to halteres increases the haltere inertial force F_i, which according to Eq. (1), increases the haltere's force in a similar fashion to their immersion in fog. Though increased drag and mass addition have very different dynamical effects, they both increase the strain registered by the campaniform sensilla embedded in the haltere shaft.

We begin by adding a large mass of glue (50–150 ng) to one haltere of a mosquito ($N = 5$). If weight is added to only one of the halteres, a mosquito can remain aloft. Presumably, it ignores the sensory feedback of the weighted haltere and maintains flight.

By weighting both halteres for each of 5 mosquitoes with a similar mass of glue, we observe they cannot fly. By drastically reducing the volume of glue, we determine the minimum amount added that precludes flight. A mass of glue $\sim 9.8 \pm 8.9$ ng ($N = 4$) added to a single haltere is at the cusp of complete loss of flight ability. At decreasing levels of glue (< 5 ng), a mosquito may still be able to remain aloft in our container, but must use the walls of its container as a guide for ascent.

With sufficient glue added, mosquitoes pitch and roll as they would in a denser-than-air gas or if both halteres had been ablated. The third column in Fig. 2 depicts a mosquito flying in normal air with glue on its halteres. The glue is shaded in blue in Fig. 4. As seen in Fig. 2(c), a mosquito with glued halteres also falls quickly.

The anomalous inertial force added to the halteres by glue is $\Delta F_i = m_{glue}A\ell(2\pi f)^2$. The mass of the glue m_{glue} is calculated based on volume estimated from images. For glue of mass $m_{glue} = 9.8$ ng, the maximum additional inertial force is $\Delta F_i = 13.6$ nN, which is similar to the haltere's aerodynamic force in fog, calculated in Section 2.2. The similarity of these two values suggest that indeed the aerodynamic force of fog on halteres is responsible for flight failure.

While the addition of mass to the halteres will do little to change $\Delta F_{D,s}$ and $\Delta F_{D,u}$, it will alter the Coriolis force measured by a rotating mosquito, thereby affecting $F_{haltere}$. The change in magnitude of the Coriolis force via glue addition may be expressed as

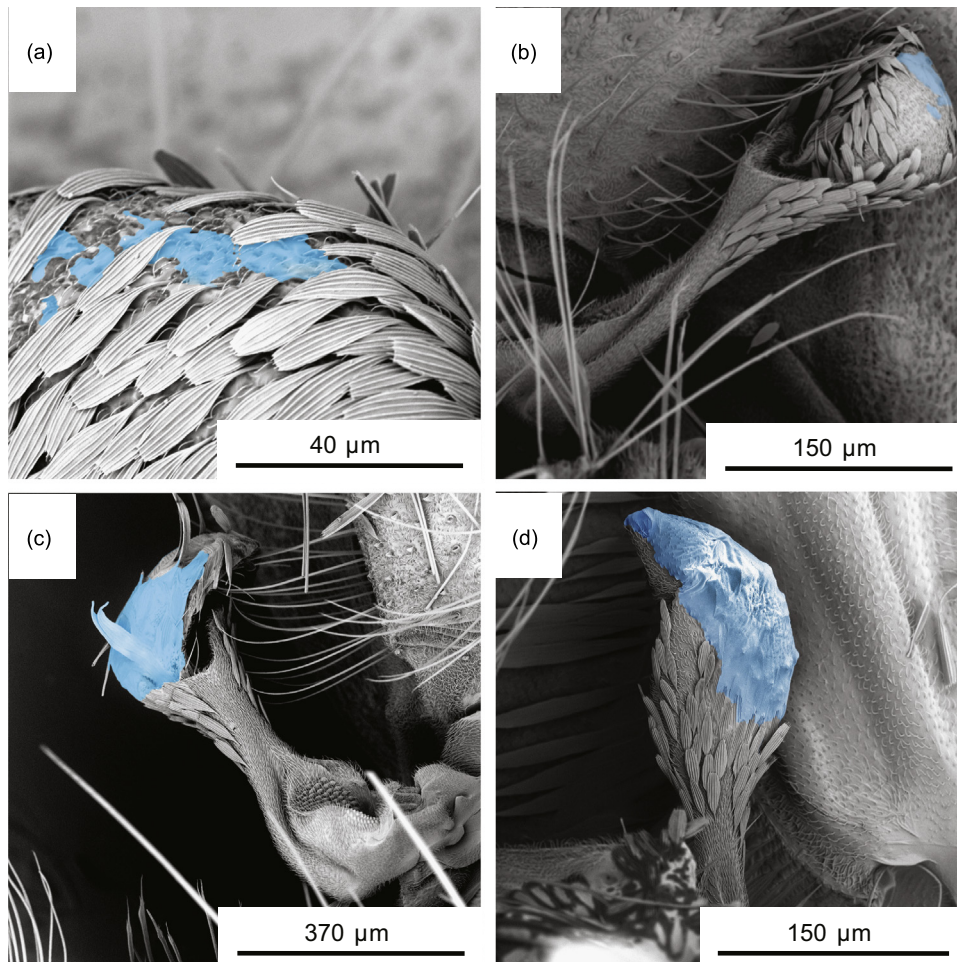$$\Delta F_C = 2m_{glue}\Omega V_{max} \tag{6}$$

Fig. 4. SEM photographs of halteres with various amount of glue, shaded in blue. The glue has a mass (a) < 1 ng, (b) 2 ng, (c) 13 ng, and (d) 20 ng. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

where Ω is the angular velocity of the mosquito's body. However, this force is small compared with ΔF_i. Consider the mosquito in Fig. 1(d), rotating at 48 rad/s, an unusually high rotation rate in our observations. Using a mass of glue of $m_{glue} = 19$ ng yields a change in Coriolis force $\Delta F_C = 1.35$ nN, which is an order of magnitude lower than the change in inertial force. Although halteres are very sensitive, it is unlikely that changes in Coriolis forces equivalent to 0.007% the weight of a mosquito have a substantial effect on flight stability.

Both the introduction of heavy gas and the treatment using glue each provide the halteres an additional resistance force. We thus pinpoint the cause of flight failure of a mosquito in fog to malfunctioning of the haltere system, and not the wings. We speculate that this anomalous force places an additional strain on the halteres during flapping. Consequently, the mosquitoes lose the ability to successfully engage steering muscles, causing them to unintentionally turn upside down. A more detailed mechanism involving the pathways from halteres to muscles is unclear. While we do not have a definitive mechanism for flight failure in hyperdense mediums, we can pinpoint the failure as a collapse in haltere function. Next, we show how the additional mass of glue can change the free vibration parameters of the halteres.

2.4. Haltere mass affects their natural frequency

All vibratory systems, physical or biological, have a natural frequency due to the geometry and elasticity of their components. Halteres are likely flapped at, or close to, their natural frequency or one of their resonance modes, to reduce the power required to reach maximum amplitude. Wings and halteres are meant to flap together, but if the vibration properties change due to haltere mass addition, a subtle mismatch in phase may occur between wings and halteres. In this section, we examine how increased mass to the haltere affects its natural frequency.

We pluck the halteres of 10 freshly dead mosquitoes, and allow them to vibrate freely (Fig. 5(a), Supp. Video 4). As seen in Fig. 5(b), the heavier amounts of glue (40–50 ng) decrease the damped natural frequency ω_d by 14% as one might expect when increasing a cantilever's inertia. Small amounts of glue (5–15 ng) increase ω_d by 15% relative to an unaltered haltere.
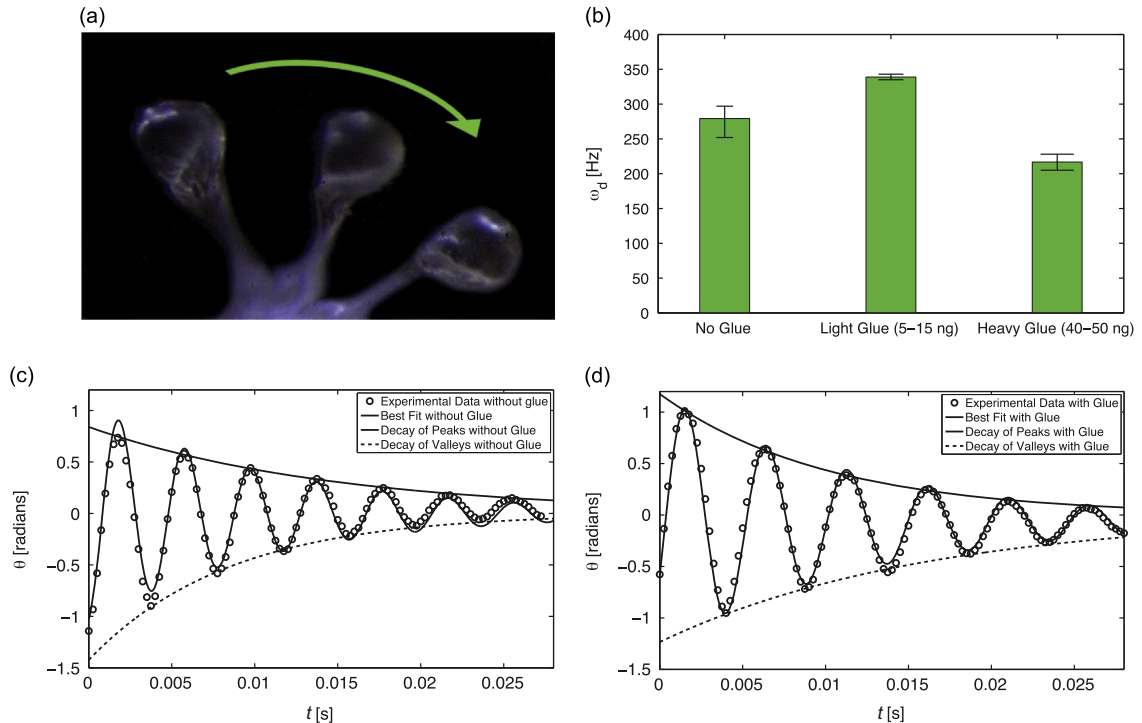
Fig. 5. (a) Haltere swinging through its plane of motion after manual plucking. (b) Damped natural frequencies of freely vibrating halteres with various amounts of glue added ($N = 8$). The bars on each column bound the minimum and maximum values for frequency. (c) Time course of freely vibrating haltere, and (d) with approximately 50 ng of glue deposited on the tip.

The cause for this increase in frequency is unknown. We find the above differences for ω_d to be statistically significant, rendering P-values of 0.02 when compared to the unaltered haltere sample.

As shown by the examples in Fig. 5(c) and (d), all of the freely vibrating halteres tested have asymmetrical exponential decay envelopes, likely the result of a single muscle which drives haltere oscillation (Pringle, 1948) alongside a thoracic oscillator (Nachtigall and Wilson, 1967) when the mosquito is flying. The lower bound of the envelope in Fig. 5(c) (dotted) levels off more quickly than the upper bound (solid), indicating preferential deflection towards the mosquito's anterior surface. The addition of glue to the haltere (Fig. 5(d)) not only decreases the resonance frequency of the haltere, from 252 Hz in Fig. 5(c) to 205 Hz in Fig. 5(d), but also slows the rate by which the vibrations decay. The changes in vibration frequencies and envelopes in plucked halteres resulting from glue addition suggest a subtle disruption to haltere kinematics in flight. Altered kinematics and mass distribution will impact strain measurements made by the haltere's campaniform sensilla, and beat rhythm, rendering halteres less effective in regulating wing motion.

3. Discussion

Our study highlights the sensitivity and precision of mosquito halteres. We induce flight failure in mosquitoes using two independent means, immersion in hyperdense flight environments and addition of mass to the halteres. Our result shows that haltere systems are highly tuned to the properties of normal air, rendering small changes to these aerodynamic properties devastating to the insect. A greater understanding of the sensitivity of such sensing systems may lead to a greater understanding of the complex control system of insects and new ways to reduce their populations.

In tests with other insects, we found that haltere size is inversely related to their vulnerability in fog. Fruitflies *Drosophila melanogaster* and *Drosophila virilis* have halteres smaller than that of a mosquito. According to our observations, they also fail to fly in dense fog. Conversely, houseflies (*Musca domestica*) with larger halteres can stay aloft in dense fog (Supp. Video 5), presumably because their flight system does not require such a finely tuned central pattern generator.

Diptera are not alone in possessing very specialized and tuned sensing organs. The male Emperor moth has chemoreceptors which can smell a female 11 km away (Taylor et al., 2004). A squid's head can detect vibration, and in the 75–100 Hz range, it can sense 0.2 µm peak-to-peak water displacement (Schmidt-Nielsen, 1997). Some snakes are able to sense vibrations of their head as small as 0.1 nm peak-to-peak amplitude. Homing pigeons can detect sound frequencies as low as 0.05 Hz, while bats can detect frequencies as high as 100 kHz (Schmidt-Nielsen, 1997). In light of the extreme sensitivity in sensing ability in the animal kingdom, disruption of mosquito haltere function with only a 2 ng addition of glue appears more feasible.

When compared to human anatomy, halteres most closely resemble the vestibular structure in our inner ear, used for balance. The inner ear is susceptible to infections, altering the inner ear's original state slightly, causing Balance Disorders (2010). The vestibular fluid in the semicircular canals of the inner ear pushes against a small membrane, the cupula, as we move our heads. It has been calculated that cupulas have a maximum displacement of 3 µm, or 0.4% the amplitude displacement of a mosquito haltere (Oman and Young, 1972). Indeed, microscopic precision in sensing, and the accompanying vulnerability, is a commonality among organisms.

Mosquitoes are not alone in being affected by extreme flight medium conditions. Altering flight medium characteristics reduces the flight performance of most flying creatures. Birds at high altitudes fly with reduced forward drag, but increased requirements for lift production (Altshuler and Dudley, 2006). Orchid bees hovering in heliox (80% He/ 20% O_2), with a density of 0.40 kg/m^3, experienced 50% increases in lift coefficients relative to normal air (Dudley, 1995). The decrease of flight ability in low density and hypoxic environments is a result of both aerodynamics and metabolism. The dynamic viscosity of the flight medium might also create instability flight. It is not possible to calculate the viscosity of our mixtures of R134a and air without further experiments (Buddenberg and Wilke, 1949), though based on the viscosities of R134a (12 µPa s) and air (19 µPa s), we do not expect the viscosity of the mixture to be dramatically different from that of air. For example, a mixture of nitrogen and R-12 (Freon) with the same volume fraction we use to a create a flight medium density $\rho = 2$ kg/m^3 (74% air, 26 % R134a) has a viscosity of 16 µPa s (Buddenberg and Wilke, 1949).

While halteres are extremely sensitive, a Reynolds number of order ten indicates that inertial effects dominate viscous effects, so changes in viscosity should have minimal effect. In the case of glue addition, the added bulk induces instability via inertial forces. Efforts were made to deposit the glue at the extreme tips of halteres, but the variation in glue mass to induce failure, $\sim 9.8 \pm 8.9$ ng, may be caused by deposition location. Haltere size variation may also play a role, and we would expect smaller halteres to be more sensitive to mass addition.

The introduction of gases other than air displaces oxygen needed for breathing and raises the possibility of hypoxia influencing the results in our experiments. At a density of 2 kg/m^3, the threshold at which no mosquito can fly, the oxygen in our container dropped from 21% by volume to 15% with the introduction of R134a. Such conditions are not prohibitive to flight: insects have been known to survive extreme hypoxic conditions (Hoback and Stanley, 2001) and colonize high altitudes above 6000 m where the oxygen content is a mere 9.5%. In our observations, mosquitoes left in containers where R134a was used to produce densities greater than ~ 3 kg/m^3 would lapse into unconsciousness within 10 s, but no sooner than 4 s. In our observations, insects will cease flapping immediately upon unconsciousness. Since the falling mosquitoes in our experiments continued flapping during descent, we are confident that hypoxia did not play a role in our results. Even at the highest gas densities, flight failure behavior occurs within a time much less than 1 s following a mosquito's drop into a container, whereas the total flight time is less than 2 s. The fast onset of flight failure and the flapping of the mosquitoes suggest that they are indeed conscious and attempting to fly in our experiments.

Due to the water repellent nature of a mosquito's body, including the halteres, fog particles are unlikely to deposit and remain attached to flapping wings, halteres, and other sensors in quantities sufficient to hinder flight. As seen in Fig. 4 the rapidly moving appendages have tightly spaced feather-like sensilla. However, a wetting liquid, such as nontoxic soybean oil (density of 920 kg/m^3), a common insecticide base, may adhere to halteres. Oil-based insecticides are known to be produced in a range of sizes (Hoffmann et al., 2009), 2–110 µm in diameter. A single oil droplet 10 µm in diameter deposited on each haltere would be sufficient to ground a mosquito. Production of insecticide-free fogs may be a viable mosquito-control strategy in some situations. We note that with current foggers, mosquitoes and similarly sized insects will be downed near the output nozzle, not due to immediate insecticide absorption, but due to the fog cloud's density.

To eliminate the likelihood of fog interrupting the function of the mosquitoes' antennae, we cropped the antennae at the base and observed that they were able to maintain qualitatively normal flight in a container. Fog exposure on mosquitoes without antennae was met with grounding, in a manner similar to those with intact organs.

Mosquito control using fog produced by ultrasonic humidifiers is not currently viable, especially for large areas. Water and electricity consumption will be unreasonable in most environments, when compared to traditional insecticide sprays. The most realistic use of water droplets to protect humans against mosquitoes would consist of a continuously falling curtain of microscopic droplets over thresholds such as windows and doors. Similar "air doors" are currently used, such that a sheet of high velocity air is directed downward from the top of a doorway. While such curtains of air are effective as an insect filter, they require high power and are noisy. In places where water is plentiful, a "fog door" would provide protection with no sound and lower operating costs.

4. Methods

Mosquito source and care: Male and female *A. freeborni* mosquitoes are used in this study, and are reared and provided by the Centers for Disease Control (CDC) in Atlanta, GA. Mosquitoes are adults upon delivery and are fed a nectar solution prepared by the CDC. We observe no difference in flight failure characteristics between male and female mosquitoes, and no attempt was made to separate the sexes following initial experiments.

Mosquito handling: Mosquitoes are transferred to various containers with a John Hock brand aspirator. They are singularly held in place by one of the two methods. The first method is by a continuous vacuum pen (Virtual Industries Tweezer Vac) which can pick up and release mosquitoes without removing appendages or rupturing their exoskeleton. The second method, used in tethered experiments, employs liquified wax to attach them to a tether, which is preferable for long-term restraint, as it places no
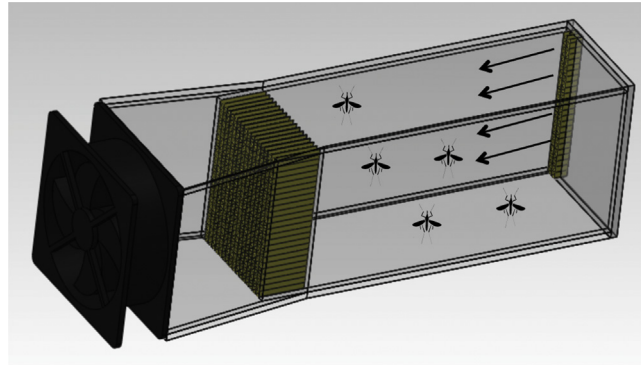
Fig. 6. Miniature wind tunnel used to visualized mosquito flight upon entering a dense fog stream and takeoff. A variable speed computer fan pulls in fog at 3 cm/s as denoted by the arrows.

external forces on the exoskeleton. Tethering with wax just behind the head or performing other tedious procedures, such as glue deposition on the halteres, requires the mosquitoes to be incapacitated by inhalation of CO_2. Short exposure to the gas anesthetizes insects, while prolonged exposure causes death. Tethering requires the mosquitoes be exposed to CO_2 for 10 s, to remain inactive for roughly 20 s.

Filming: A Phantom V210 high-speed camera is the primary tool for observing of mosquito flight, and we film over a range of 3000–10,000 fps. A Nikon AF Nikkor 50 mm 1:18D lens is used to capture the entire flight arena, while a Navitar 1-60135 is used for macro filming. Flight arenas are lit by 4, low temperature LED's (IDT Honeycomb LED-1). Measurement and tracking within videos is done with Tracker, an open source physics program. All reported *P*-values in this paper are results of an unpaired *t*-test application to our data.

Fog experiments: Fog is produced with an Air O Swiss 7145 consumer humidifier with continuous adjustability in fog density. A hose attached to the humidifier directs the stream of mist to the subject. A droplet sizing instrument (DC-III; KLD Labs Inc., New York, NY) is used to characterize the spectra of droplets generated by our humidifiers. As seen in Fig. 6, a miniature wind tunnel (7.5 cm $\times$ 5 cm $\times$ 11 cm) has been constructed to deliver fog to a mosquito's contained, flight environment. It uses a variable speed computer fan to pull air through a 6.5 cm $\times$ 1 cm opening, which uses a square grate to laminarize the incoming air and contain mosquitoes. The air speed at the inlet is 3 cm/s, measured by particle tracking. Control experiments indicate no substantial effect of incoming air on mosquito flight. Flight is evoked from the mosquitoes within by human breath into the tunnel's intake. Human breath excites both male and female *A. freeborni*.

Dense Gas Experiments: For experiments with flight in various gas densities, R134a (1,1,1,2-Tetrafluoroethane), a non-toxic gas (Alexander and Libretto, 1995), with a density of 4.25 kg/m^3 at standard temperature and pressure, is piped into a sealed, transparent, semi-circular container of height 15 cm and width 5.5 cm. Mosquitoes were capable of indefinite perching on the interior walls and ends of the container post-experiment. Subjects used in R134a-based experiments are still alive the day following exposure to R134a, when sufficient ambient oxygen is allowed into their container. The density of the gas mixture inside the container is determined by the container's mass reading on an analytical balance. Atmospheric conditions inside our lab remain with 5 °C range. Mosquitoes were introduced through a hole in the top of the container, and allowed us to fly freely, or fall to the bottom.

Haltere augmentation experiments: To investigate the effect of altered forcing on mosquito halteres, we laden the halteres of untethered, anesthetized mosquitoes with UV curable glue with density $\rho_{glue}=960$ kg/m^3, the haltere tip. The glue's density is measured by massing a disc of cured glue whose volume is measured by digital calipers. The glue is deposited onto unconscious mosquitoes via a thin wire, by hand, under a microscope. Glue is cured with a UV flashlight. Mosquitoes are given 10 min to recover from anesthesia and released for observation. Mosquitoes anesthetized by the same manner as those with glue on halteres recovered to resume qualitatively normal flight after approximately 5 min in atmospheric air. Post-flight, mosquitoes are imaged in a Phenom Pro G2 scanning electron microscope (SEM), which provides a reference scale in frame, to measure the volume of glue deposited. Glue dollops are traced with Tracker to calculate the irregular shape's area and thickness.

5. Conclusion

We perform flight experiments with mosquitoes in mediums of varying fluid density. In gases with densities twice that of standard air, mosquitoes suffer rapid pitching, rolling, and ultimately grounding. Though no directly applicable fluid dynamic model exists, we estimate drag forces on the halteres flapping in dense air using steady and unsteady drag models, which provide comparable values. We hypothesize the anomalous aerodynamic drag forces, on the order of 10 nN, disrupt the rhythm of mosquito halteres, their gyroscopic sensors and wingbeat pattern generators. We test this hypothesis by adding weight to the halteres to create inertial forces on the same order of magnitude as aerodynamic forces in heavy air. An increase in the haltere's mass by 10 ng is sufficient to disable flight. We also show that weighted halteres have different

natural frequencies than unaltered halteres, and suggest that this change in natural frequency prevents synchronization between wings and halteres. This study shows that halteres are highly tuned to the properties of air, making changes in air density a potential method to be applied in mosquito control.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this paper can be found in the online version at http://dx.doi.org/10.1016/j.jfluidstructs.2015.03.016.

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