A Comparative Study of Dragonfly Flight

in Variable Oxygen Atmospheres

by

Joanna Randyl Henry

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Graduate Supervisory Committee:

Jon F. Harrison, Chair
Alexander Kaiser
Ronald L. Rutowski

ARIZONA STATE UNIVERSITY

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ABSTRACT

One hypothesis for the small size of insects relative to vertebrates, and the existence of giant fossil insects, is that atmospheric oxygen levels have constrained body sizes because oxygen delivery would be unable to match the needs of metabolically active tissues in larger insects. This study tested whether oxygen delivery becomes more challenging for larger insects by measuring the oxygen-sensitivity of flight metabolic rates and behavior during hovering for 11 different species of dragonflies that range in mass by an order of magnitude. Animals were flown in 7 different oxygen concentrations ranging from 30% to 2.5% to assess the sensitivity of their behavior and flight metabolic rates to oxygen. I also assessed the oxygen-sensitivity of flight in low-density air (nitrogen replaced with helium), to increase the metabolic demands of hovering flight. Lowered atmosphere densities did induce higher metabolic rates. Flight behaviors but not flight metabolic rates were highly oxygen-sensitive. A significant interaction between oxygen and mass was found for total flight time, with larger dragonflies varying flight time more in response to atmospheric oxygen. This study provides some support for the hypothesis that larger insects are more challenged in oxygen delivery, as predicted by the oxygen limitation hypothesis for insect gigantism in the Paleozoic.

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INTRODUCTION

Why are insects so small compared to vertebrates? Several possible explanations have been proposed. Smaller body sizes may be adaptive due to competition with or predation by birds, reptiles, and mammals (Damuth, 1981; Blackburn and Gaston, 1994). Alternatively, some nonadaptive mechanistic constraints on insect size may occur. The exoskeletons of insects may not be able to support large bodies due to scaling problems (Price, 1997), and/or the lack of anaerobic capacities may enforce an upper size limit due to reduced maximal power output (Marden, 1994). In recent years, the finding that insect gigantism in the late Paleozoic was correlated with atmospheric hyperoxia has stimulated the hypothesis that atmospheric oxygen levels have constrained the size of insect because oxygen delivery would be unable to match the needs of metabolically active tissues in larger insects (Graham *et al.*, 1995). One possible prediction from this hypothesis is that oxygen delivery should be more challenging for larger insects; therefore, a higher partial pressure of oxygen (P_{O_2}) in the atmosphere would allow larger insects to exist and function.

The experiments that have been conducted to test the hypothesis that insect body size is constrained by oxygen availability have yielded mixed results. Some single- (Louden, 1989; Greenburg and Ar, 1996; Frazier *et al.*, 2001; Peck and Madrell, 2005) and multi- generation studies (Henry and Harrison, 2004) have shown that there is a positive correlation between insect mass and rearing oxygen level. Nevertheless, other studies have shown that larger insects are less sensitive to severe hypoxia when resting (grasshoppers: Greenlee and Harrison,

2004; Greenlee *et al.*, 2007), hopping (grasshoppers: Kirkton *et al.*, 2005), or feeding (caterpillars: Greenlee, 2005). However, perhaps the inverse size effect on oxygen sensitivity occurred because these prior studies tested insects at rest when oxygen consumption rates are low.

During insect flight in normoxia, oxygen consumption rates increase dramatically (10-100-fold) while, in hypoxia, and safety margins for oxygen delivery decline (Rascón and Harrison, 2005; Harrison *et al*., 2006). Another serious critique of these prior tests of whether larger insects have smaller safety margins for oxygen delivery is that most of these (except Greenlee *et al.*, 2007) have been intraspecfic studies. Variation in all parameters, including size, increases in cross-species comparisons.

This interspecific study looks at the effects of body size on the sensitivity of dragonfly hovering flight to atmospheric oxygen level. The only prior study of oxygen delivery in a flying dragonfly found that hyperoxia stimulated flight metabolic rate, suggesting that these animals are highly sensitive to oxygen (Harrison and Lighton, 1998). Ventilation during flight of dragonflies is thought to occur primarily by autoventilation (Weis-Fogh, 1967), so perhaps this group is more sensitive to changes in atmospheric oxygen than groups that rely heavily on abdominal pumping, which can be decoupled from wing movements. The largest fossilized insects (Protodonata) were morphologically very similar to dragonflies, so perhaps had similar tracheal morphologies and ventilatory mechanisms. I measured behavioral and physiological responses to changes in ambient oxygen concentrations using multiple species of dragonflies that vary by an order of

magnitude in body size. Using flow-through respirometry, the animals' flight metabolic rates were measured in 2.5, 5, 7.5, 10, 15, 21 and 30% O_2, balanced with N_2 (nitrox). Animals were also flown in hypodense air using helium as a balance gas (heliox). The use of hypodense air increases the power requirements and metabolic rates during flight (Dudley, 1998; Roberts *et al*., 2004). Safety margins for oxygen consumption during flight may be reduced by the higher metabolic rates during flight in hypodense air; but increased by higher diffusion rates for oxygen in lower-density air. If larger dragonflies are more sensitive to changing oxygen levels, then I predicted that I would observe a positive relationship between dragonfly mass and the response of flight performance to changing oxygen levels.

METHODS

Animals and Study Sites

Eleven species of dragonflies (Table 1) were collected from the Soda Springs Desert Studies Center at Zzyzx, California, which is located at the western end of the Mojave National Preserve (35°08'35" N: 116°06'15" W). A man-made, spring-fed pond (Lake Tuendae) supports at least 13 species of dragonflies (Polcyn, 1994). Since different dragonfly species emerge as adults during different summer months, I conducted three collecting trips—one in July 2004, one in May 2005 and a final trip in August 2005. Oxygen effects on flight metabolic rates and performance were measured within 5 min of collecting the animal, at an outdoor respirometry set-up located next to Lake Tuendae. The wet body mass (±0.001g) of all captured animals was measured using an analytical balance (Mettler AE100; Mettler Toledo, Columbus, OH, USA). Roughly half of the species captured tended to patrol the lake continuously throughout the day categorizing them as "fliers" while the remaining species, categorized as "perchers", preferred to sit on vegetation and only flew when actively hunting or attempting to mate.

Experimental Design

The power of interspecific comparative analyses depends on the number of species used, and in this case, on the range of masses (Harvey and Pagel 1991). Because many measurements were made on individuals (flight behavior and metabolic rate in 14 different gas mixes plus some additional controls), several hours were required to complete measurements for a single individual. In

addition, the hours that dragonflies are easily available are relatively limited, necessarily limiting the number of animals that could be assessed. Thus I decided to focus on obtaining the maximum number of species, and our study had relatively low number of individuals per species (Table 1). Unless otherwise stated, reported values are species means (averaging across individuals of that species for each parameter).

Individual dragonflies were captured with a net or by hand, and transferred to the clear plastic flight chamber, which was also a flow-through respirometry system (Figure 1). A 4 L chamber was used which allowed unimpeded free flight, which generally produces higher metabolic rates than tethered flight (Kammer and Heinrich, 1978); though some species did occasionally fly into the chamber walls. After allowing 5 min for the dragonfly to equilibrate to the experimental atmosphere, a video-recording of the flight chamber was initiated, and flight was induced by gently shaking the flight chamber. CO_2 released during flight activity was recorded (Figure 2). If animals did not fly, the chamber was shaken relatively continuously for at least two min. The flight performance (number of flight bouts, flight duration, total flight time) and CO_2 emission rates were measured in test gases of 2.5, 5, 7.5, 10, 15, 21 and 30% oxygen, with the balance being either N_2 (nitrox) or helium (heliox). Each animal was flown in all of the gas mixtures; however, the order in which the animal was exposed to the differing oxygen levels was randomly determined. Duration of the "flight test" for each test gas was approximately 3 min. A subset of animals was flown in normoxia before switching to each new test gas to test for degradation in performance over time.

The barometric pressure at Zzyzx during the course of the experiment was 101.07 ± 0.04 kPa (286 m elevation).

Video Analysis of Behavior

The video camera's angle of view encompassed the entire flight chamber; a flight bout was defined as a continuous period of time when the wings were active and animals were off the chamber floor. Flight bout durations were measured using of frame-by-frame analysis of video taken by a digital video recorder (ZR series; Canon, New York, USA). Video images were shot at a standard rate of 30 frames per second; thus, bouts of activity as short as 0.03 sec could be detected.

Respirometry

The flow rate of air through the flight chamber (constant at16.2 ± 0.1 L min^{-1}) and the oxygen concentration of the mixture were regulated by mass flow controllers and meters (Omega, Stamford, CT, USA). The flow rate was chosen to reduce washout effects and improve temporal resolution (95% equilibration time was less than one minute) while keeping flows low enough so that the CO_2 output of the excurrent air could be accurately determined. Our oxygen analyzer was not sufficiently precise to measure oxygen consumption rates during flight; the oxygen readings were instead used to confirm the gas mixes. Excurrent air from the chamber was dumped into a manifold from which the air was subsampled at a rate of 500 ml min^{-1}. The subsampled air was first dried (magnesium perchlorate) and then pulled sequentially through a CO_2 analyzer (LI-6252; Li-Cor, Lincoln, NE, USA), Ascarite II (for CO_2 removal), and then an O_2 analyzer

(FOXBOX , Sable Systems, Las Vegas, NV, USA) by a pump (R-1; AMETEK, Pittsburg, PA, USA). The output of both analyzers was digitized and recorded using Sable Systems DATACAN (Las Vegas, NV, USA).

The metabolic rates during flight were calculated by integrating the area under each CO_2 emission peak (Figure 2) that corresponded to a burst or closely-timed burst of flight, and dividing by the time spent in flight as determined using the video recording of behavior.

The flight chamber was housed in a temperature-controlled environment to reduce the effects of temperature on metabolic rate. The temperature was maintained at 31.6 ± 0.1°C by monitoring the temperature within a 0.76 m x 0.76 m x 0.91 m wood-framed, Plexiglas chamber and adjusting the output from an attached air conditioner accordingly.

Statistical Analyses

I tested for general effects of oxygen and air density on our dependent parameters (flight CO_2 emission rate, number of flight bouts, flight bout duration, total flight time during the flight test) using a general linear analysis using oxygen, air density, and body mass as independent factors. I first tested for three-way interaction terms, and then two-way interaction terms.

To assess the oxygen-responsiveness of dragonflies, I plotted the dependent variables vs. oxygen for each species and calculated the linear slope. I then tested whether these slopes (oxygen-responsiveness) were statistically related to body mass using linear regression.

Because observed differences in metabolic rate and behavior may be affected by phylogenetic relatedness in addition to physical size differences, phylogenetically independent contrasts (PICs) were calculated for each of the independent and dependent variables used in this study using the ape package in R (Paradis, *et al.*, 2004; Felsenstein, 1985).

To calculate the PICs, a supertree that included all of the species tested at Zzyzx was constructed by combining two other trees (Saux, 2003; Ware, 2007) using a strict supertree algorithm (Figure 3; Sanderson, *et al.*, 1998). A second tree was generated that assumes that all libellulid species were equally related to each other in a monophyletic clade, while keeping the aeshnids separated. A third tree was constructed using random branch lengths and relatedness.

All statistical analyses were carried out using R language (R Core Development Team, 2010; http://www.R-project.org/); graphs were generated using the ggplot2 and lattice packages (Wickham, 2009; Sarkar, 2008). Results were determined to be significantly different from the null hypothesis by using an experimental type I error less than or equal to 5%. Analysis of covariance (ANCOVA) and linear regressions were used in the analysis of mass effects on metabolic rates and flight behaviors. All values are shown as means ± S.E.M. unless otherwise noted.

RESULTS

Effect of flight bout duration on CO_2 emission rates

Many of the flight bouts were quite short (a few seconds), raising the question of whether the CO_2 emission rates approximated steady-state conditions. To assess this question, I tested the relationship between flight bout duration and the measured carbon dioxide emission rate associated with that peak in CO_2 emission rate for individual animals flown in 21% nitrox. Although CO_2 production rates were more variable when flight bout durations were short, they were independent of flight bout duration, suggesting that even the very short flight bouts of a few seconds approximate steady-state flight (Figure 4). CO_2 emission scaled non-significantly with mass to the 0.44 power. Though the regression model was a poor fit, this slope did not significantly differ from the 2/3 power relationship between mass and metabolic rate typically seen in insects (t-test on slopes: $t=0.071$, d.f.=9, $p>0.05$).

Air density effects on flight metabolism and behavior

Dragonflies consistently produced carbon dioxide at a higher rate when hovering in hypodense atmospheres (heliox mixtures, Figure 5, Table 2). CO_2 emission rates averaged about 10% higher when the dragonflies were flown in heliox, but in 2.5% oxygen atmospheres, CO_2 emission rates were 75% higher than in nitrox. The flight bouts were also significantly more frequent in heliox gas mixtures (Figure 6, Table 3). However, there was no consistent or significant effect of air density on flight bout duration or total flight duration (Tables 4 and 6).

Oxygen effects on flight metabolism and behavior

There was an overall effect of oxygen concentration on CO_2 emission rates (Table 2). However, at oxygen concentrations equal or greater than 5%, carbon dioxide production remained relatively constant; while at 2.5% oxygen, metabolism rates drop (Figure 5, Table 2). All measures of flight behavior were highly significantly affected by oxygen level (Tables 3-5), with a general positive correlation between flight behavior and oxygen level (Figures 6-8).

The default flight behavior of each species did not alter the oxygen-dependent changes to CO_2 production or observed flight behavior since slopes of perchers and fliers were not significantly different (Figure 9).

Body mass effects on flight metabolism and behavior

The size of the dragonfly species did not significantly affect carbon dioxide production in this study, but it did affect measures of flight behavior (Tables 2-5). Larger dragonflies generally flew longer (in oxygen levels greater than 10%) and less often than smaller species (across all oxygen levels; Figures 10-12).

To assess response to oxygen levels within species, I plotted the value for each dependent variable vs. oxygen and calculated the slope of a linear regression, assessing nitrox and heliox data separately (Figure 13). Then these slopes were plotted vs. mass to test whether larger animals were more responsive to varying oxygen level. The response of CO_2 emission rate to oxygen level was not statistically related to body mass. However, the response of total flight duration to atmospheric oxygen in nitrox atmospheres was greater in larger dragonflies

(Figure 14, slope = 0.03 * log mass + 0.01, R^2=0.39, p=0.04). The oxygen-responsiveness of total flight duration in nitrox was also significantly related to body mass when tested with phylogenetic corrections using both the supertree and the two clade tree (Supertree: slopes = 0.04 * log mass + -0.002, R^2=0.50, p=0.02; Aeshnids vs Libellulids: slopes = 0.04 * log mass + 0.01, R^2=0.51, p=0.02; Figure 14). However, randomly generated trees eliminated the significant relationship between oxygen-responsiveness of flight duration and body mass (Figure 14).

Flight bout number and flight duration tended to show a similar effect (greater responsiveness in larger insects, all slopes were positive), but these were not statistically significant. In contrast, slopes of the oxygen-responsiveness of flight behavior and CO_2 emission were not significantly related to body mass in heliox.

DISCUSSION

My study is one of the first to test oxygen-sensitivity during aerobic flight across multiple species. Observed oxygen responsiveness for flight metabolic rate was independent of mass, suggesting that hovering flight performance was relatively invariant. However, larger dragonflies showed a stronger correlation between oxygen level and the total duration of flight, indicating that the concentration of oxygen does influence either the commencement or termination of flight behavior. Since the effects on behavior match our predictions, these data do provide new evidence that larger insects can be more responsive to changes in atmospheric oxygen.

Reliability of the flight CO_2 emission rates

The low sample size used in this experiment likely contributed to the low power of tests and resulted in poorly fit regressions when calculating the mass-scaling of metabolic rate in different oxygen levels. Despite this, there was a clear positive relationship between the mass of these dragonflies and their carbon dioxide emission rates and the slope found in this student (0.44) was not significantly different from similar slopes found in other studies looking at insect metabolic rates (Niven and Scharlemann, 2005). A number of other factors could also contribute to the lower than expected correlation between mass and metabolic rate. Dragonflies may have lower wingbeat frequencies compared to other insects of similar size resulting in lower metabolic rates or the larger dragonfly species captured may not have been flying optimally in the small

chamber used in this experiment thereby reducing the observed correlation between mass and CO_2 production.

Flight durations were quite short in this study (averaging 2.86 ± 0.36 sec) since most of the dragonfly species (though able to hover) were not willing to maintain steady flight in the flight chamber. Harrison and Lighton (1998) measured much longer flight durations; however, they chose their target species specifically for its long duration flights in a respirometer. This raises the question of whether the flight CO_2 emission rates could be measured accurately, and represented steady-state conditions. Unlike vertebrates, insects rely almost exclusively on aerobic metabolism to fuel flight (Beenakkers *et al.*, 1985) therefore even very short flight bursts would be expected to show some CO_2 production. However, ATP stores within flight muscle cells are low often requiring the use of arginine phosphate as a temporary substrate for ATP production. Arginine phosphate can also act as an energy shuttle moving phosphate rapidly between the mitochondria and myofibrils (Schneider *et al.*, 1989) which could account for the nearly instantaneous increase in insect flight metabolism that is typically observed.

I found no relationship between flight duration and flight CO_2 emission rate (Figure 4), suggesting that even flights of a few seconds represented steady-state conditions. In addition, assuming a respiratory exchange ratio of 1 indicative of a carbohydrate-based metabolism, the flight metabolic rates recorded in this study (0.05 to 0.38 Watts) were similar to reported values for *Erythemis simplicicollis* and *Sympetrum sanguineum* (0.12 Watts and 0.02 Watts

respectively; Harrison and Lighton, 1998; Wakeling and Ellington, 1997). My estimates of metabolic rate for *Anax junius* (0.134 Watts) were lower than those reported by May (0.37 Watts; 1995); however, that study used thorax temperature during flight to estimate power generation. Also, May showed that these dragonflies decreased wingbeat frequency, thorax temperature, and had lower metabolic rates as ambient temperature increased (1995). My animals were consistently flown at a high temperature of 30°C while the highest temperature recorded in May's study was 26.5°C (1995).

Oxygen-sensitivity of flight metabolism and behavior

Since most animals were able to fly in oxygen levels of at least 5%, dragonflies seem to adequately deliver oxygen to metabolically active tissues regardless of size. This conclusion differs from the findings for a single species of dragonfly, *Erythemis simplicicollis* (Harrison and Lighton, 1998). For this species, it was found that hyperoxia stimulated CO_2 emission rates, though flight behavior did not differ from normoxia. I suspect that this difference may relate to the relatively low power of this study to detect changes in CO_2 emission rate in response to oxygen. The single-species study of Harrison and Lighton (1998) measured 25 individuals at each oxygen level, compared to 1-4 individuals per species in this study, leading to a much greater power to detect significant, within-species changes in flight parameters with oxygen.

The high temperature used in this study may have also served to inhibit flight performance as animals attempted to reduce thorax temperatures (May, 1995). Since maximal flight performance might not have been achieved,

regardless of atmospheric density, changes in behavior should be a better index of the sensitivity of these animals to hypoxia.

Inducing maximal flight power output by using hypodense air

Dudley (1995) demonstrated the power production in Orchid bees can increase up to 45% when flown in a hypodense normoxic atmosphere. This method of forcing insects to work harder has many benefits compared to the alternatives traditionally chosen. Attaching weights or tethering the insect may alter an insect's center of gravity or might reduce the animal's motivation to fly.

Roberts (2004) used this same method to study the kinematics and metabolic rates of Carpenter bees. In this case, Roberts maintained normoxic conditions, but altered the proportion of He to N_2 in his balance gasses. Only the smallest bees in his study were capable of achieving flight in the 100% heliox mixture; this gas mix is 0.8 kg ml^{-1} less dense than 100% nitrox.

In my study, balance gas significantly affected metabolic rate and the number of flight bouts (Figures 5 and 6), but did not play a role in the other measures of flight behavior. This suggests that the high concentration of helium in the balance gas improved diffusive gas exchange to the tissues. In fact, the gas-phase diffusion coefficient of oxygen in helium is 3.67 times greater than in nitrogen (Timmons, 2000).

Oxygen delivery as a factor limiting body size in insects?

Overall, the behavior of larger odonates tended to be more oxygen-sensitive than that of smaller species (Figure 14). In particular, the total time spent in flight was significantly reduced for larger animals exposed to low oxygen

levels in normodense atmospheres. This evidence supports the hypothesis that highly active insects may be more responsive to changes to atmospheric oxygen, which, in turn, may have been a major contributing factor in limiting insect size.

To add to this body of evidence, more work could be done to investigate the ventilatory mechanisms utilized by dragonflies during flight. While flight behavior was sensitive to oxygen level, flight metabolic rates were not. Unlike vertebrates, insects demonstrate a proportional investment in respiratory structures (via tracheal hypermetry) as body size increases (Greelee, *et al.*, 2009; Lease, *et al.*, 2006; Kaiser, *et al.*, 2007). By looking at the tracheal morphology of dragonflies using similar methods, it should be possible to determine if the flight metabolic rates' insensitivity to oxygen was due to an overbuilt respiratory system in larger dragonflies.

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Table 1

Species	Number of Animals	Mass (g)	CO_2 Emission Rate (μmol/hr)	Mean Flight Duration per Bout (sec)	Total Flight Time (sec)	Number of Flight Bouts
Aeshna multicolor **F**	1	0.6338	350.29	4.54	13.61	3
Anax junius **F**	1	1.2329	1015.44	4.77	38.13	8
Libellula Comanche **P**	1	0.3882	1206.54	2.74	10.95	4
Libellula luctuosa **P**	3	0.2847 ± 0.06 (0.1558 – 0.3597)	348.93 ± 127.32 (128.66 – 569.71)	1.36 ± 0.12 (1.12 – 1.50)	11.49 ± 4.50 (2.9 – 18.08)	9 ± 3
Libellula saturata **P**	3	0.4311 ± 0.03 (0.3767 – 0.4521)	1210.80 ± 273.15 (719.71 – 1663.60)	4.23 ± 1.82 (1.75 – 7.78)	34.49 ± 24.62 (5.24 – 85.54)	6 ± 2
Macrodiplax balteata **P**	1	0.2189	582.10	2.21	11.03	5
Pachydiplax longipennis **P**	4	0.1631 ± 0.02 (0.1105 – 0.2020)	475.96 ± 115.72 (209.04 – 765.91)	2.75 ± 1.86 (0.89 – 4.61)	11.9 ± 6.54 (5.36 – 18.44)	5 ± 1
Pantala flavescens **F**	3	0.1496 ± 0.03 (0.0913 – 0.1970)	474.91 ± 207.87 (207.07 – 884.19)	1.40 ± 0.18 (1.25 – 1.75)	10.05 ±3.41 (6.26 – 16.85)	8 ± 3
Pantala hymenaea **F**	1	0.2997	2909.53	2.57	25.67	10
Tramea lacerata **F**	1	0.4387	742.13	2.95	8.86	3
Tramea onusta **F**	2	0.3534 ± 0.003 (0.3508 – 0.3559)	660.64 ± 290.64 (370.00 – 951.27)	1.90 ± 0.31 (1.59 – 2.21)	8.58 ± 4.16 (4.42 – 12.73)	5 ± 3

Table 1. Summary table showing the mean masses, CO_2 emission rates, and behavior characteristics for each species flown in 21% oxygen balanced with nitrogen. S.E.M. values and ranges are included if multiple animals from the same species were tested. **F** = flier; **P** = percher

Table 2

Source of Variation	Degrees of Freedom (Df)	Sum of Squares (SS)	Mean Squares (MS)	F-value	P-value
Density	1	2.061	2.061	4.242	**0.041**
Oxygen	1	14.897	14.897	30.663	**<0.001**
Mass	1	0.167	0.167	0.344	0.558
Total	149	72.386	0.486		

Table 2. ANOVA table demonstrating the impact of gas density, oxygen content, or species body mass on $\log_{10} CO_2$ emission rate.

Table 3

Source of Variation	Degrees of Freedom (Df)	Sum of Squares (SS)	Mean Squares (MS)	F-value	P-value
Density	1	5.503	5.503	4.705	**0.032**
Oxygen	1	46.860	46.860	40.060	**<0.001**
Mass	1	9.951	9.951	8.507	**0.004**
Total	150	175.462	1.170		

Table 3. ANOVA table demonstrating the impact of gas density, oxygen content, or species body mass on the number of flight bouts (√N).

Table 4

Source of Variation	Degrees of Freedom (Df)	Sum of Squares (SS)	Mean Squares (MS)	F-value	P-value
Density	1	0.002	0.002	0.412	0.522
Oxygen	1	0.070	0.070	13.054	**<0.001**
Mass	1	0.102	0.102	19.091	**<0.001**
Total	134	0.714	0.005		

Table 4. ANOVA table demonstrating the impact of gas density, oxygen content, or species body mass on mean flight duration (log_{10} sec).

Table 5

Source of Variation	Degrees of Freedom (Df)	Sum of Squares (SS)	Mean Squares (MS)	F-value	P-value
Density	1	0.025	0.025	3.6937	0.057
Oxygen	1	0.340	0.340	50.226	**<0.001**
Mass	1	0.031	0.031	4.641	**0.033**
Total	135	0.900	0.007		

Table 5. ANOVA table demonstrating the impact of gas density, oxygen content, or species body mass on total flight duration (log_{10} sec).

Figure 1

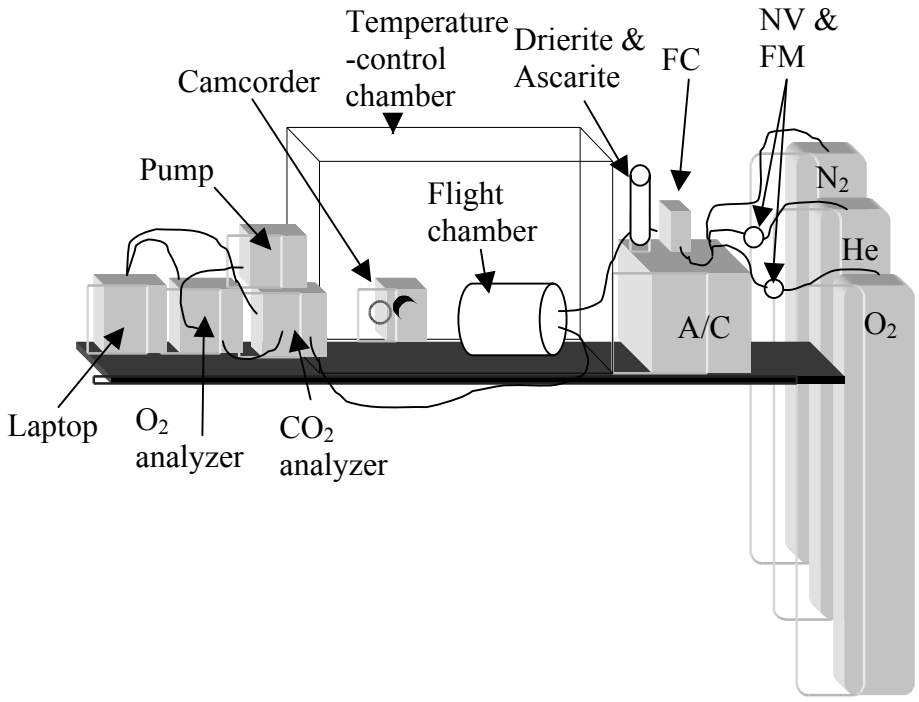


Figure 1. Schematic of experimental setup in the field. FC = mass flow controller, NV = needle valves, FM = flow meters. N_2, He, and O_2 indicate compressed air cylinders with dual-stage regulators, and A/C is the air conditioning unit that was used to regulate the temperature of the temperature-box.

Figure 2

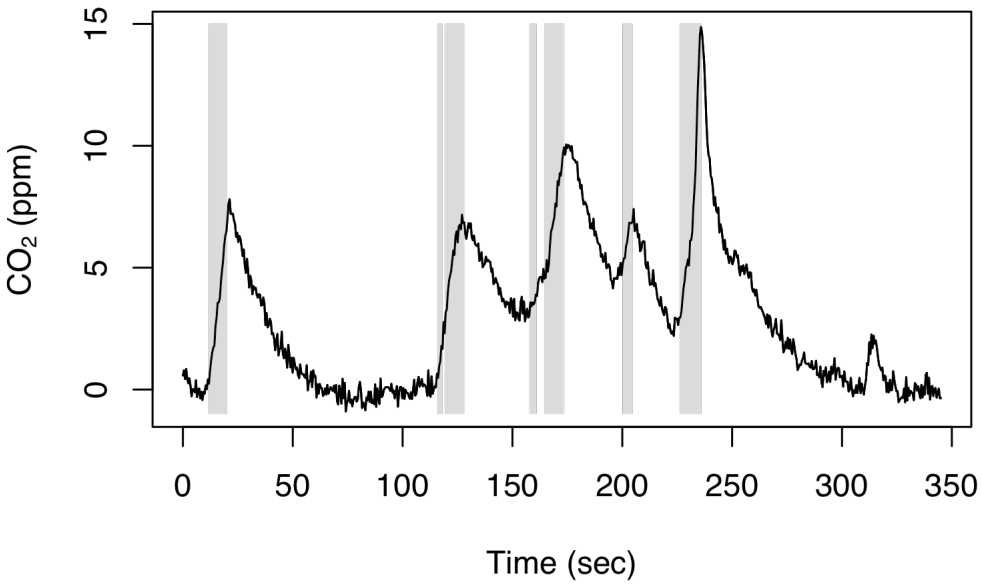
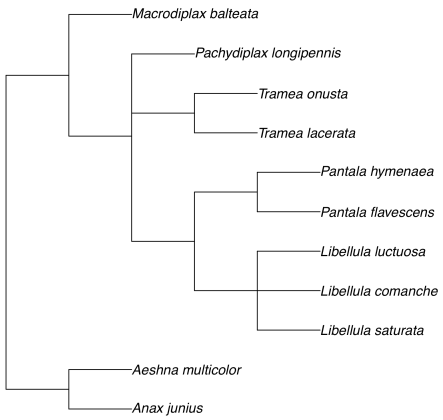


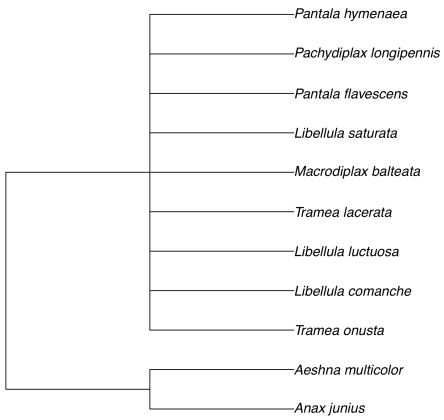
Figure 2. Representative trace of the carbon dioxide content of excurrent air sampled from the flight chamber. This particular example shows the CO_2 produced by the darner, *Anax junius*, in 30% nitrox. Each flight bout duration is indicated by the width of the light gray bars.

Figure 3

A.



B.



C.

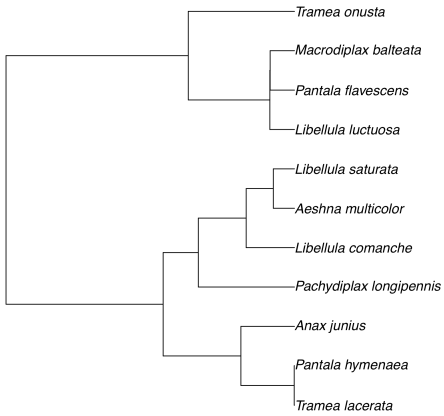


Figure 3. Three phylogenetic trees used to calculate PICs: A) supertree, B) simple tree dividing dragonflies into two clades, C) random relation and branch lengths.

Figure 4

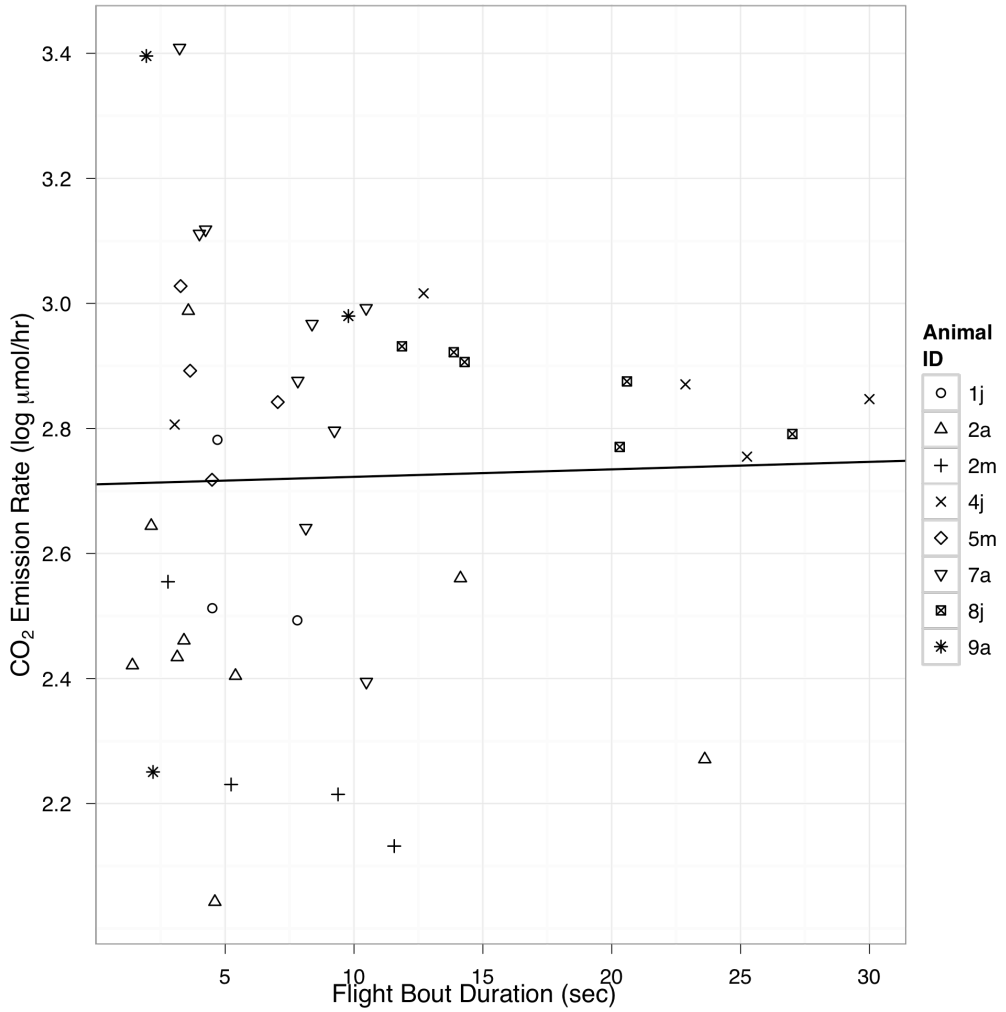


Figure 4. Carbon dioxide emission rate was independent of the duration of flight bouts. To simplify visualization, only 8 animals are shown. The regression was not significantly different from zero (a = 0.001, b = 2.711, $F_{(1,41)} = 0.034$, p = 0.855, $R^2 = 0.001$).

Figure 5

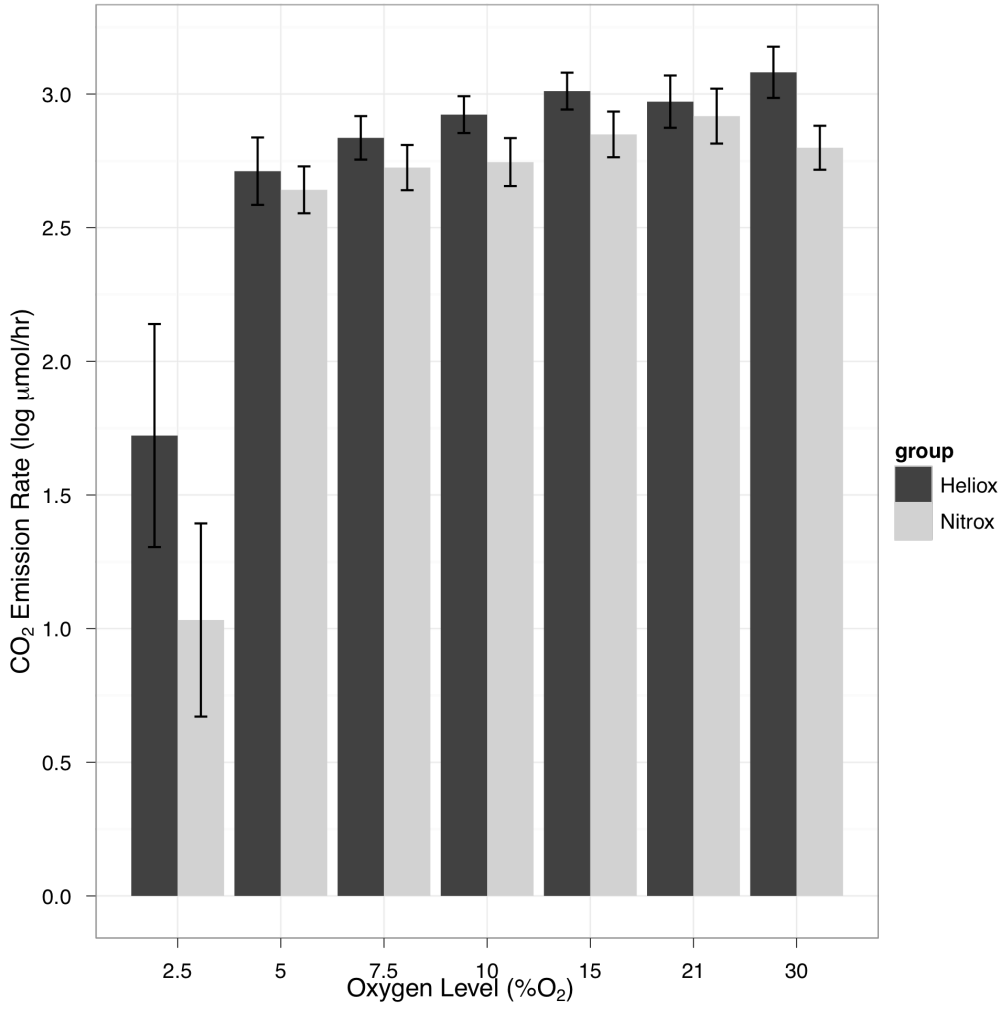


Figure 5. Carbon dioxide emission versus oxygen level. Dark bars represent mean emission rates in hypodense air and light bars represent mean emission rates in normodense air.

Figure 6

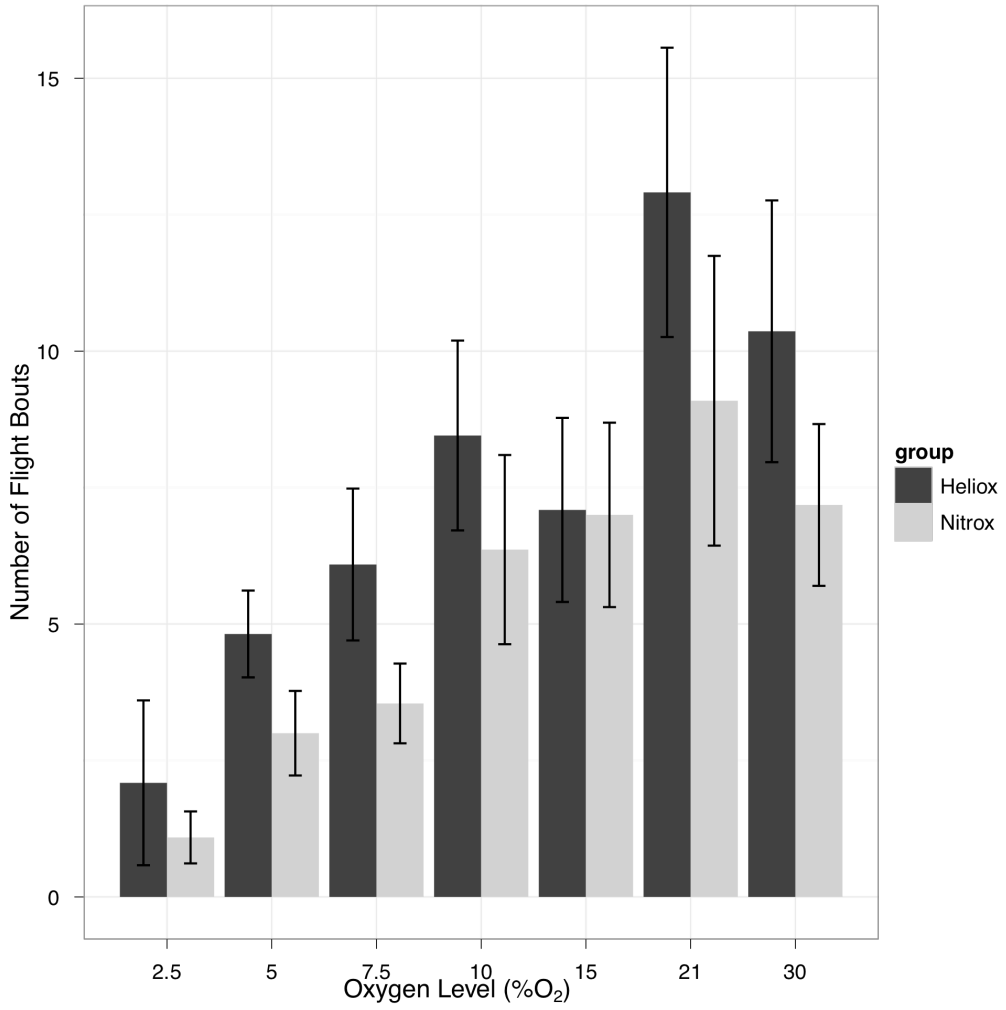


Figure 6. Frequency of flight versus oxygen level. Dark bars represent the number of flight initiated in hypodense air and light bars represent the number of flights in normodense air.

Figure 7

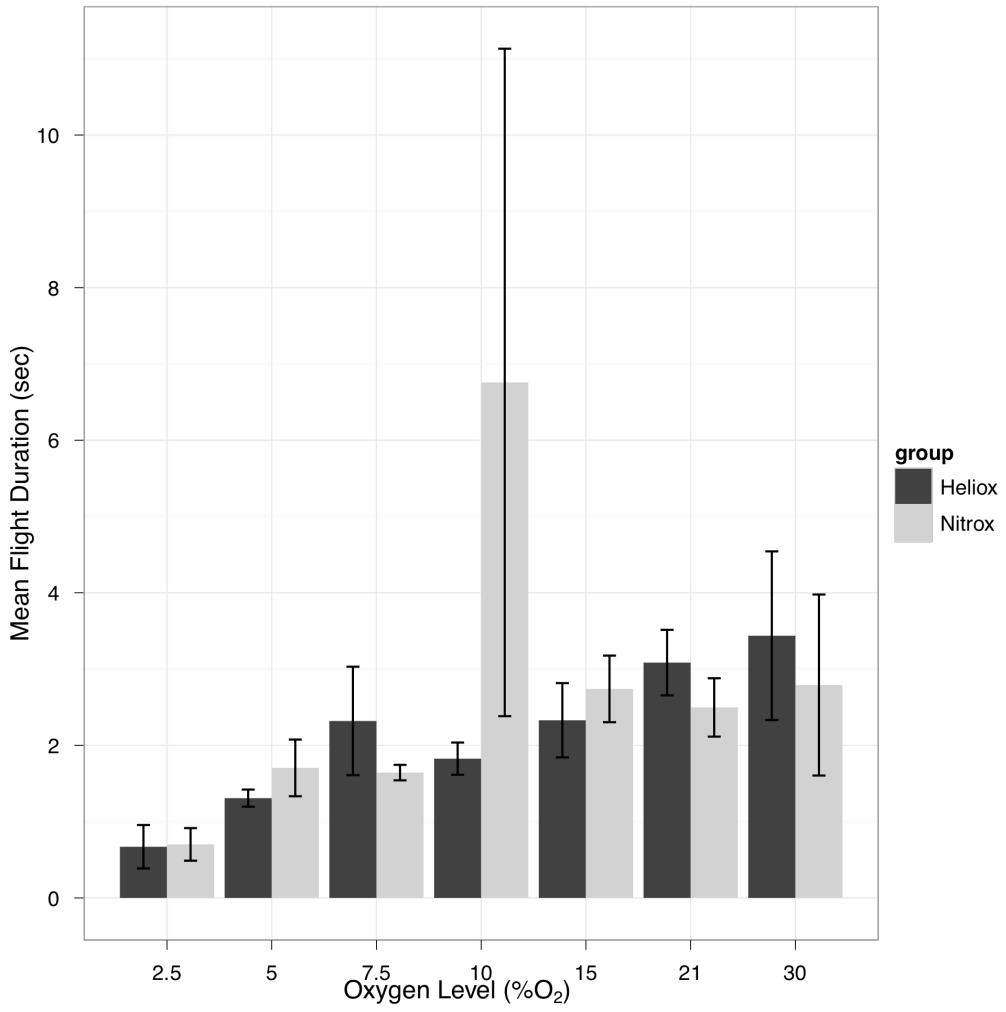


Figure 7. Mean flight duration versus oxygen level. Dark bars represent flight times in hypodense air and light bars represent flight times in normodense air.

Figure 8

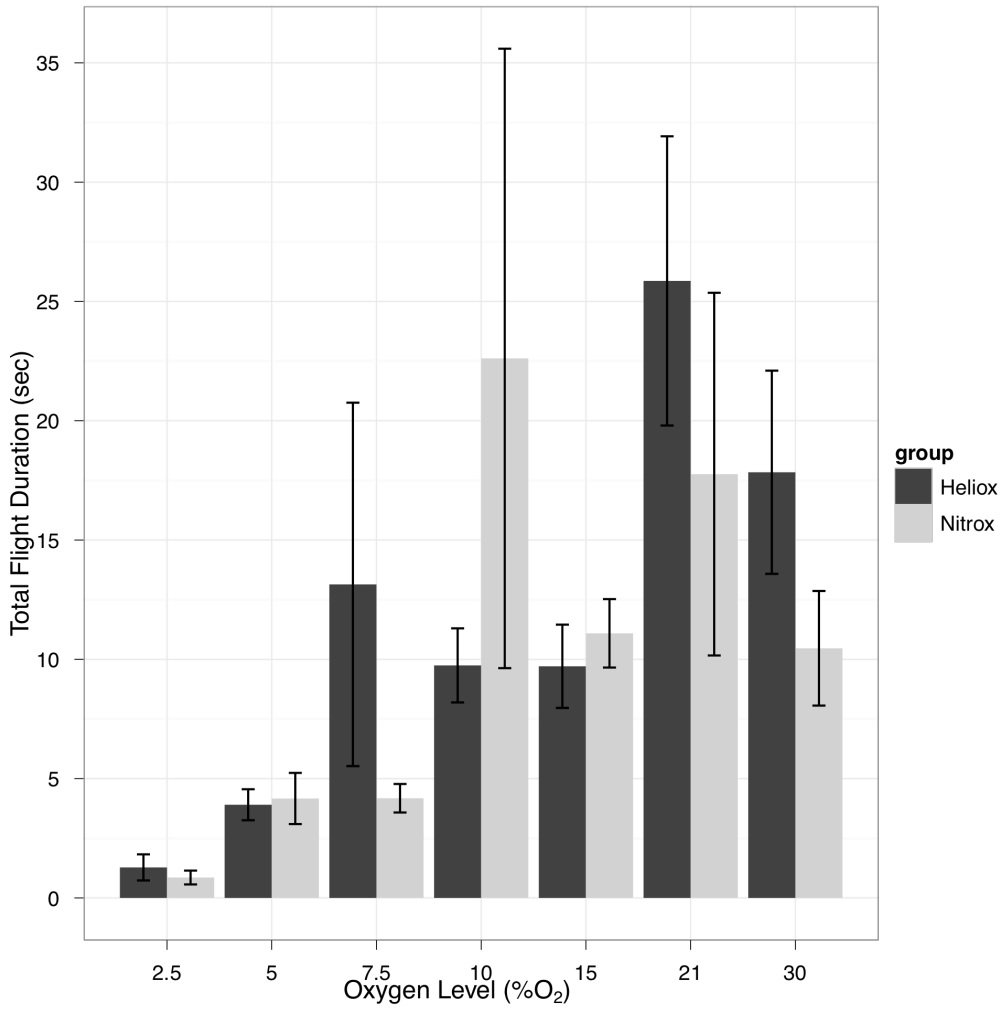


Figure 8. Total flight duration versus oxygen level. Dark bars represent the total time spent in flight in hypodense air and light bars represent flight times in normodense air.

Figure 9

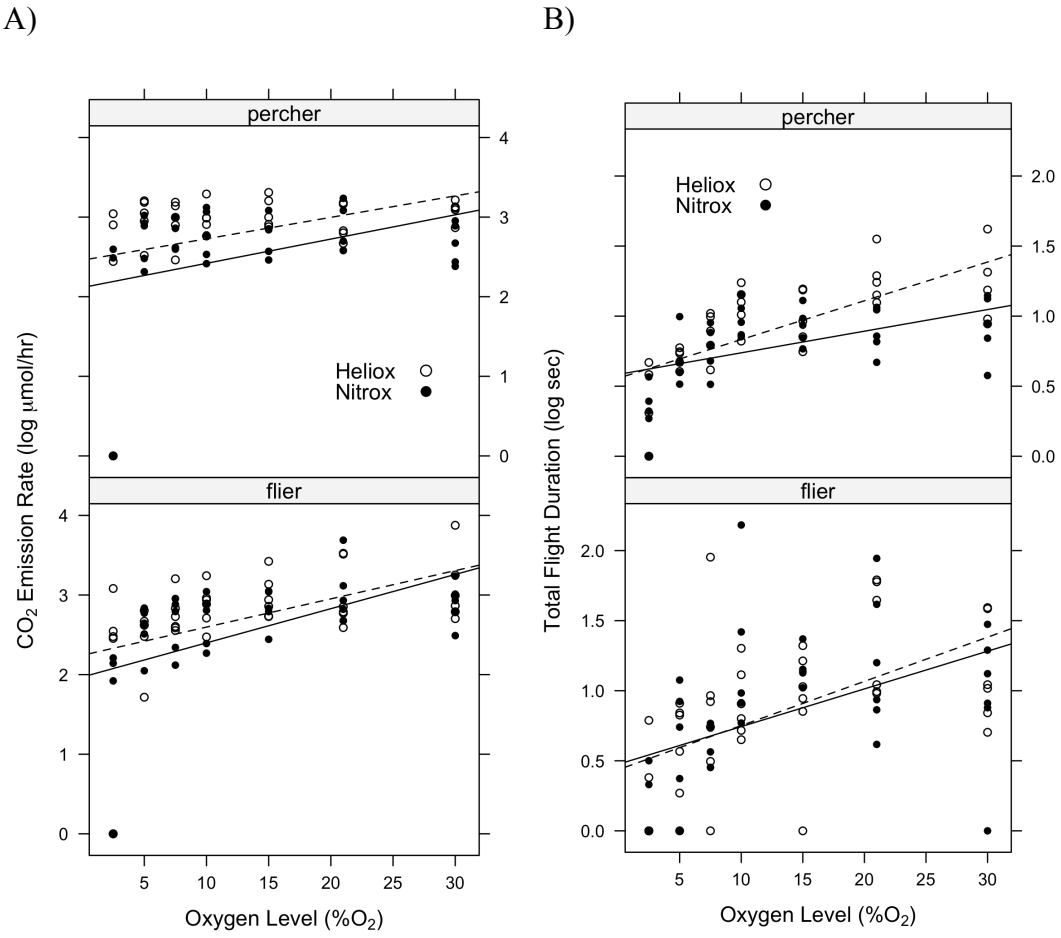


Figure 9. Regressions of A) CO_2 emission rate and B) total flight duration versus oxygen level. Default flight behavior (percher or flier) did not significantly affect the dependent variables.

Figure 10

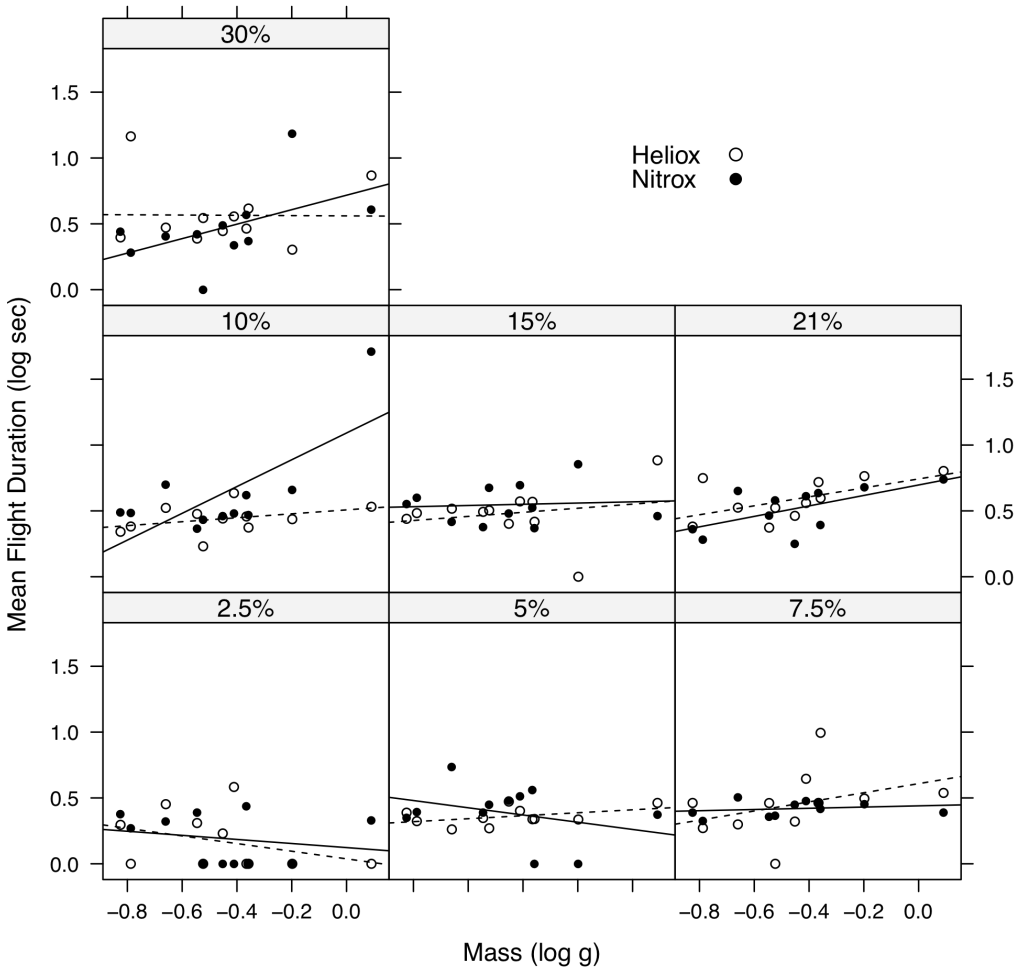


Figure 10. Mass scaling of flight duration within each oxygen concentation.

Figure 11

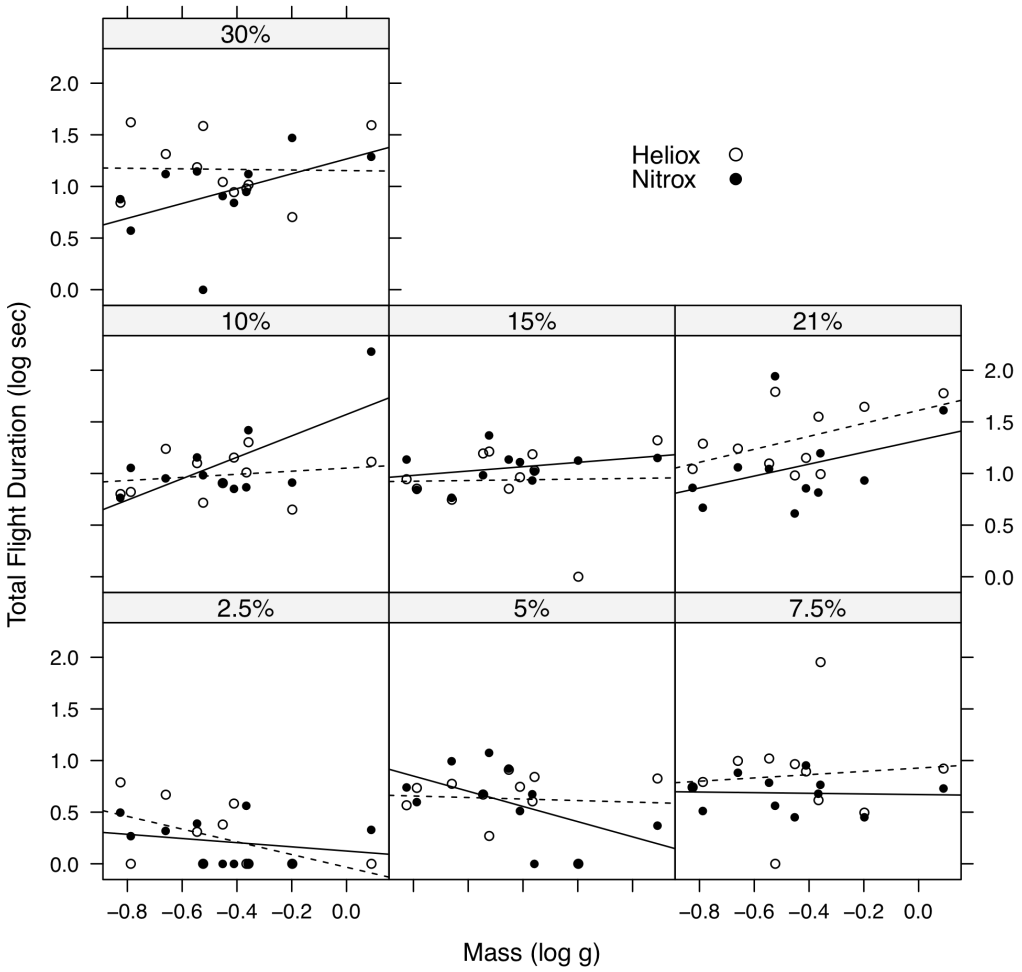


Figure 11. Mass scaling of total flight duration within each oxygen concentation.

Figure 12

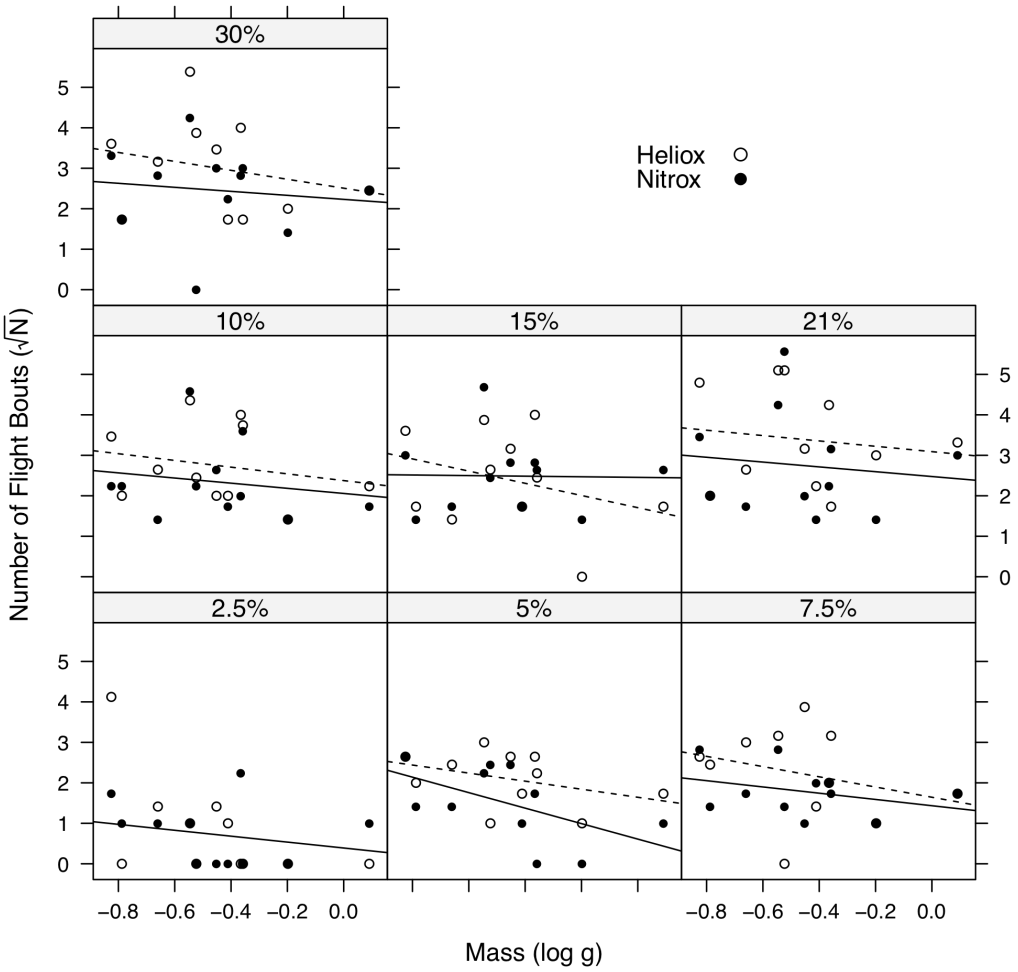


Figure 12. Mass scaling of flight bout frequency within each oxygen level.

Figure 13

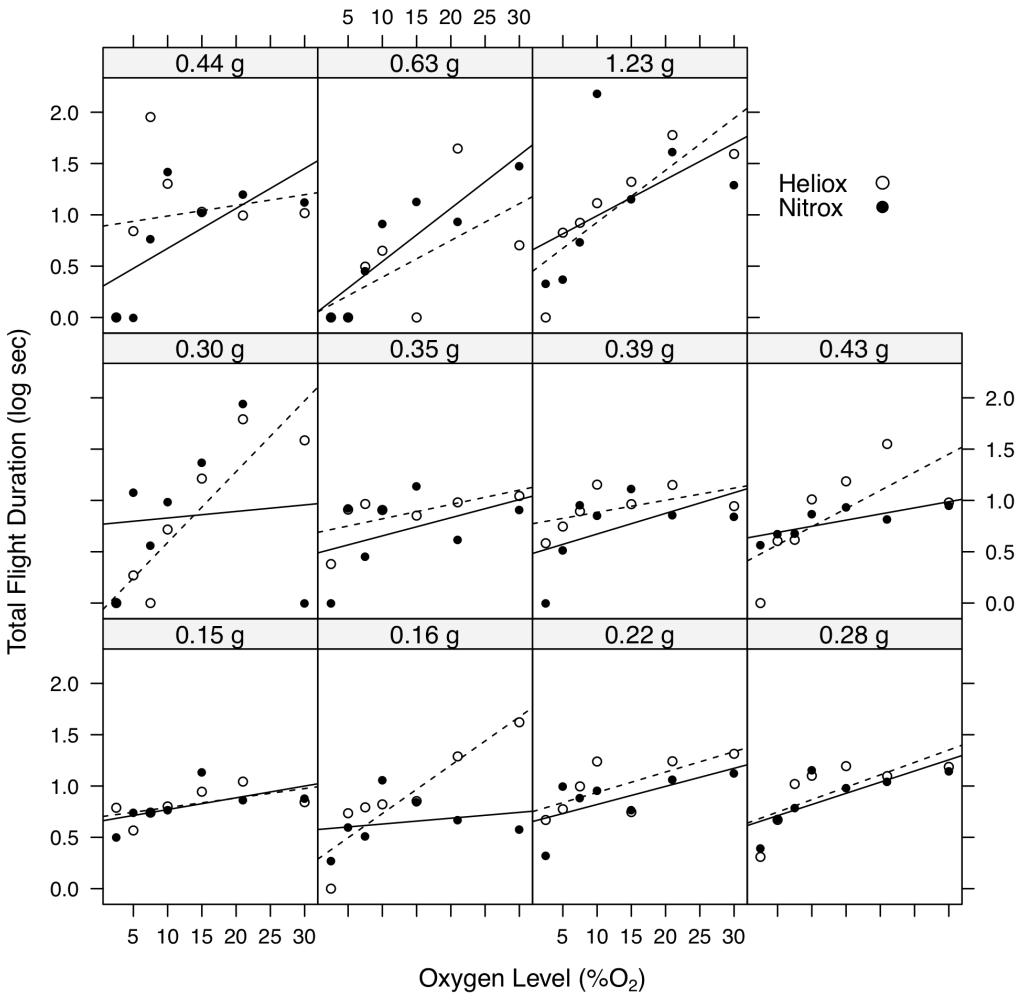


Figure 13. Representative graph showing the linear regressions performed within each species. The resultant slopes from each graph was recorded and used to test if there was an interaction between each dependent variable and mass.

Figure 14

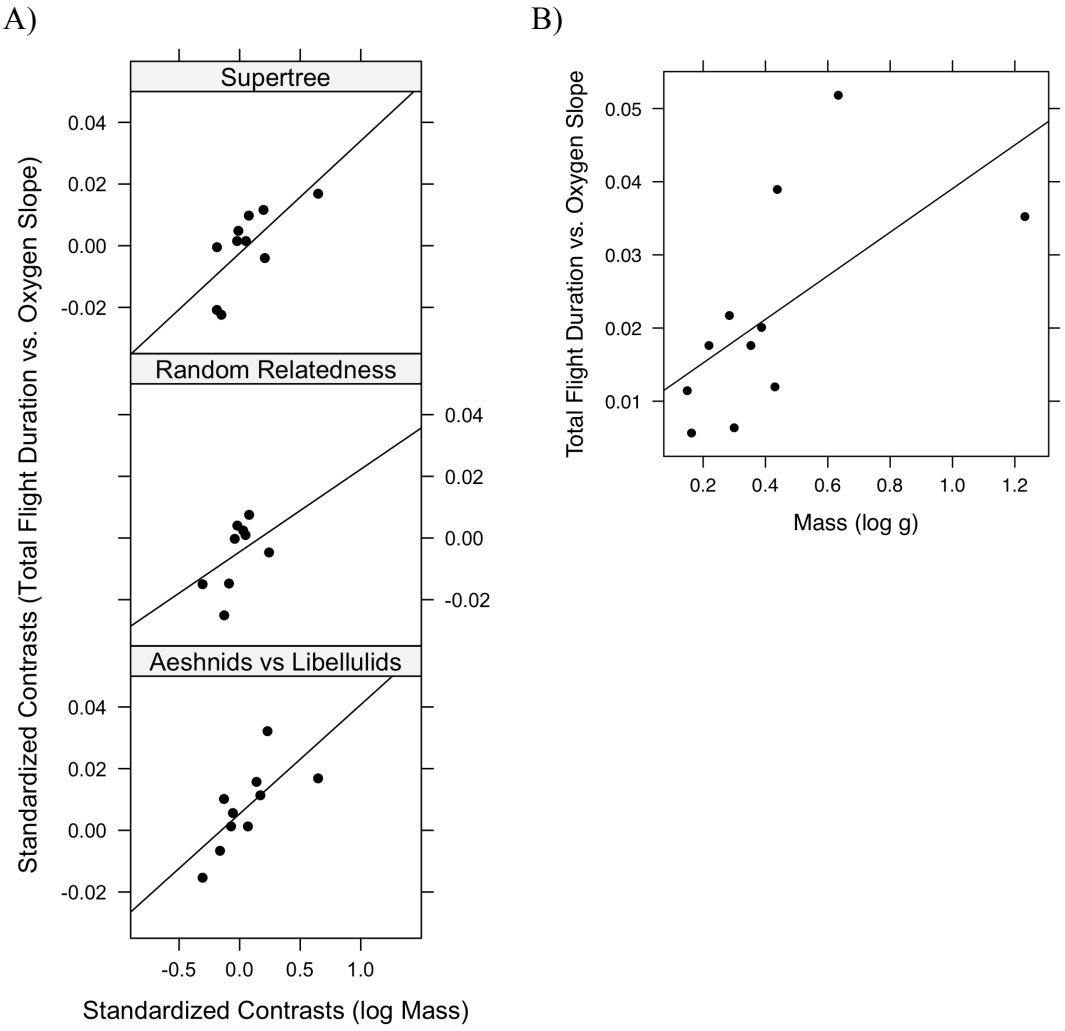


Figure 14. Visual representations of mass interactions with oxygen on total flight duration in nitrox. A) Regressions generated using phylogenetically independent contrasts derived from alternate phylogenies. (Random Relatedness: slope = 0.03, $R^2 = 0.97$, $p < 0.001$; Supertree: slope = 0.04, $R^2 = 0.50$, $p = 0.022$; Aeshnids vs Libellulids: slope = 0.04, $R^2 = 0.51$, $p = 0.020$) B) Regression calculated using phylogenetically uncorrected data (slope = 0.03, $R^2 = 0.39$, $p = 0.041$).