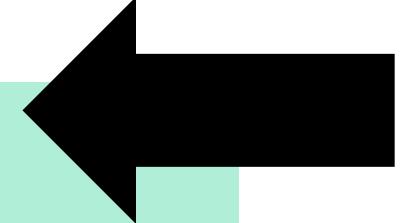


# Flight Performance of *Jadera haematoloma* as a Function of Temperature, Relative Humidity, and Age

Anastasia Bernat

# Two Main Test Groups

- 
1. Testing Field Bugs (Preliminary Extreme Trials)
  2. Testing 1 Generation Down of the Field Bugs (Age-Controlled Extreme Trials)
  3. Timeline & Materials

# Preliminary Trials

*A priori* information:

Min temperature (T) in Florida = 18.8 C (Tallahassee, Feb),

Min relative humidity (RH) = 47% (Orlando, April, afternoon)

Max temperature (T) in Florida = 43 C (Monticello, June),

Max relative humidity (RH) = 95% (Gainesville, September, morning )

Source: [Florida Climate Center](#). Temperature Normals & Relative Humidity.

250-300 bugs (20-30 from each pop – 8 populations total) arriving August 13

After bugs are tested for the standard trials, field collected bugs will be tested under this regime: **Temperature treatments at 12, 20, 36, 44 C (at 70% RH). Humidity treatments at 40, 55, 85, 100% (at 28 C). 14:10 (L:D).** There are 8 testing regimes. The last testing regime from the standard flight trials will also apply here (i.e. 28 C, RH 70%).

# Preliminary Trials

After bugs are tested for the standard trials, field collected bugs will be tested under this regime: **Temperature treatments at 12, 20, 36, 44 C (at 70% RH). Humidity treatments at 40, 55, 85, 100% (at 28 C). 14:10 (L:D)**. There are 8 testing regimes. The last testing regime from the standard flight trials will also apply here (i.e. 28 C, RH 70%).

- Can do 60 bugs per treatment, each treatment on different days. Ideally, equal number of males and females. Half mainland, half keys populations. \*
- If these numbers are too high, I can do 30 bugs per treatment where I'm only comparing the sexes or mainland vs. keys. (Choose one sex or one population). \*

Stop testing at 4 PM each day.

\* Selection of each subset is randomized



~8-16 days

# Preliminary Trial Methods

1. Day before:
  - I. Test up to 60 field bugs per treatment. Select equal number of males and females, half mainland and half keys, randomly.
  - II. Generate datasheet(s) the day before.
  - III. Check paint on bugs.
  - IV. Give Gatorade picks.
  - V. Leave bugs in 28 C and 70% RH.
2. Testing Day:
  - I. Set incubator to a treatment (e.g. 12 T and 70% RH).
  - II. Record mass and which females laid eggs.
  - III. Give bugs 10-15 minutes to acclimate to the temperature.
  - IV. Test up to 60 bugs in one day for that treatment. Follow the same experimental set-up used in standard flight trials.
  - V. Count how many eggs a tested female laid and collect eggs if necessary.
  - VI. Place tested bugs in the fridge and then in alcohol (except selected eye-laying females).
  - VII. End flight testing at 4 PM each day
3. Repeat steps 1-2 for a new treatment (8 treatments total)

# Preliminary Analyses

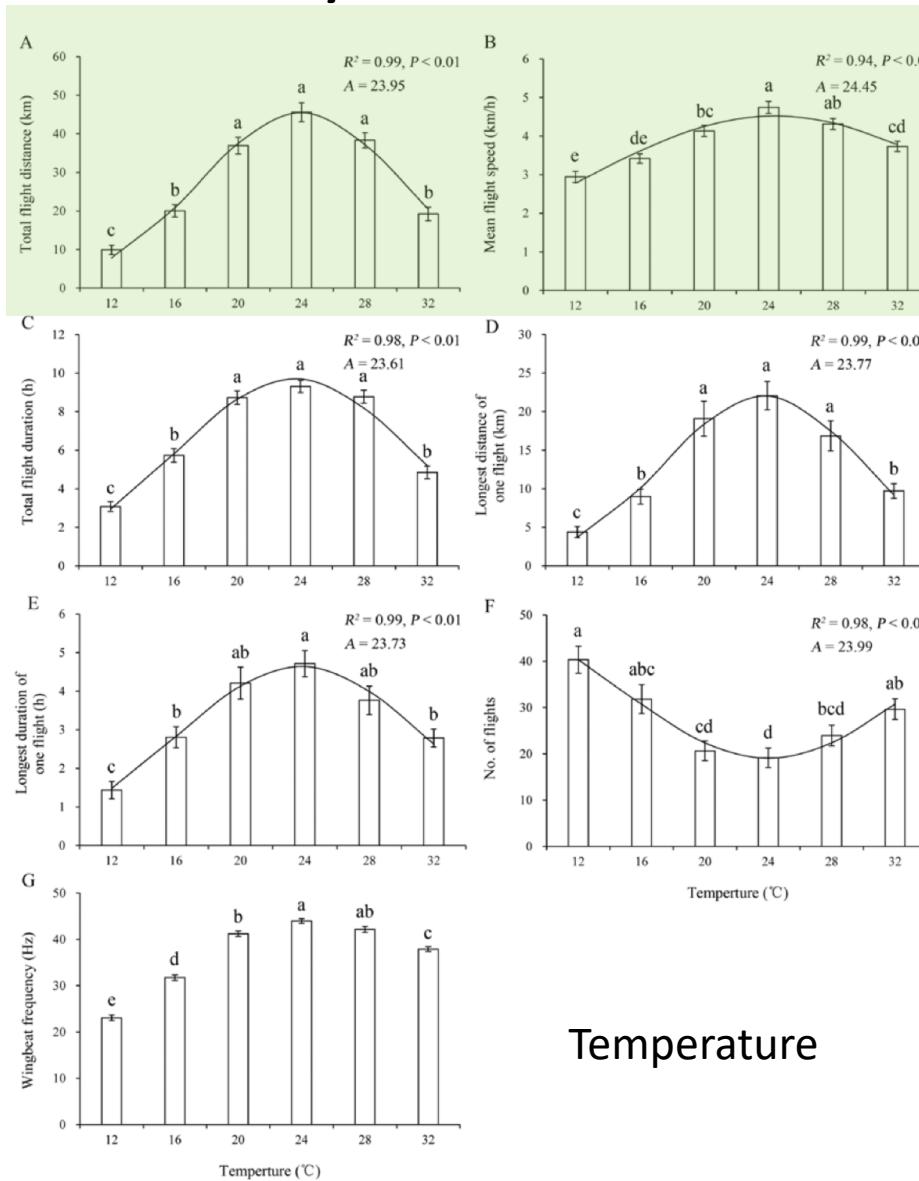
Run one-way ANOVA and nonparametric tests (Kruskal-Wallis, Wilcoxon test, Tukey's HSD test) on flight parameters (speed, distance) for different testing regimes (standard vs. temp and RH settings).

Run binomial and Gaussian glm models to determine which factors are significant in predicting speed, distance, or yes-no flight.

If strong differences exist on field collected bugs, then I have a strong enough reason to believe that they will impact the flight behavior of lab raised bugs. The age of lab raised bugs will be recorded so as to separate age effects from temperature and RH effects.

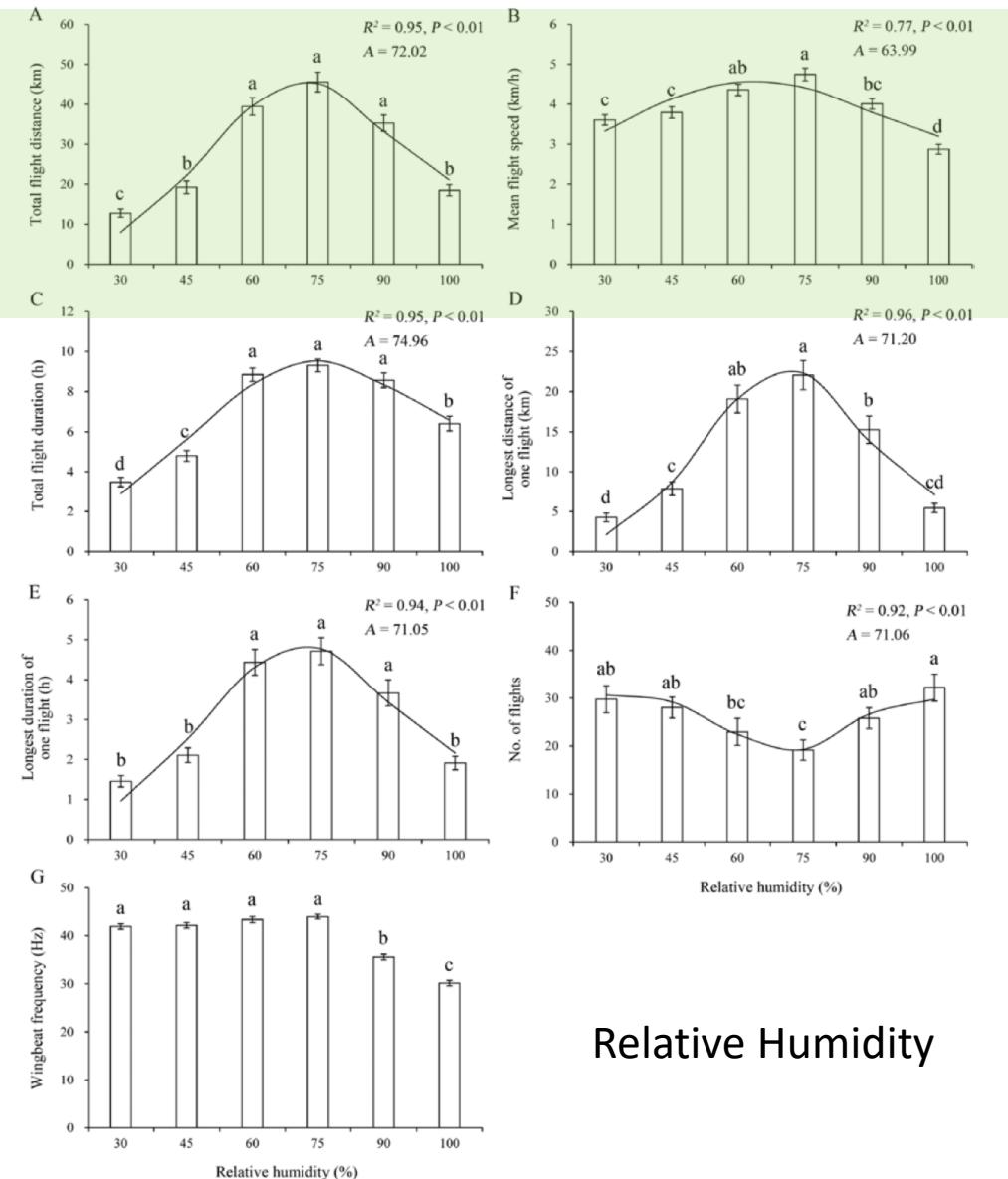
# Preliminary Ex Results

## Total Distance



Temperature

## Avg Speed



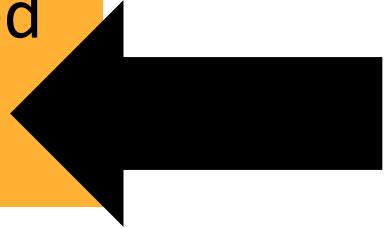
Relative Humidity

# Two Main Test Groups

1. Testing Field Bugs (Preliminary Extreme Trials)

2. Testing 1 Generation Down of the Field Bugs (Age-Controlled  
Extreme Trials)

3. Timeline & Materials



# Age-Controlled Trials

During flight trials, need to collect eggs in an organized and intentional way. That is because need a set of bugs whose age is known and kept track of.

Age Groups: Young, adult, old (but with specific days)

Age groups need to be tested on the same day. If I test 30 bugs a day conservatively then have 10 bugs per age group. 5 males, 5 females per age group. These numbers are low and it might be ideal to double them. Or reach 48 or 54 bugs.

One mother (minimize genetic variability) or several mothers

Which mother to take eggs from? The ideal would be having a mother that reaches 60 eggs that hatch at different times. In case that is not enough, I will follow three mothers in total and collect them assuming that each will lay 60 eggs at different times. Any that do not will be eliminated from the experiment and who ever is left will be randomly chosen.



~2 months  
to raise lab-  
SBB

# Age-Controlled Trials

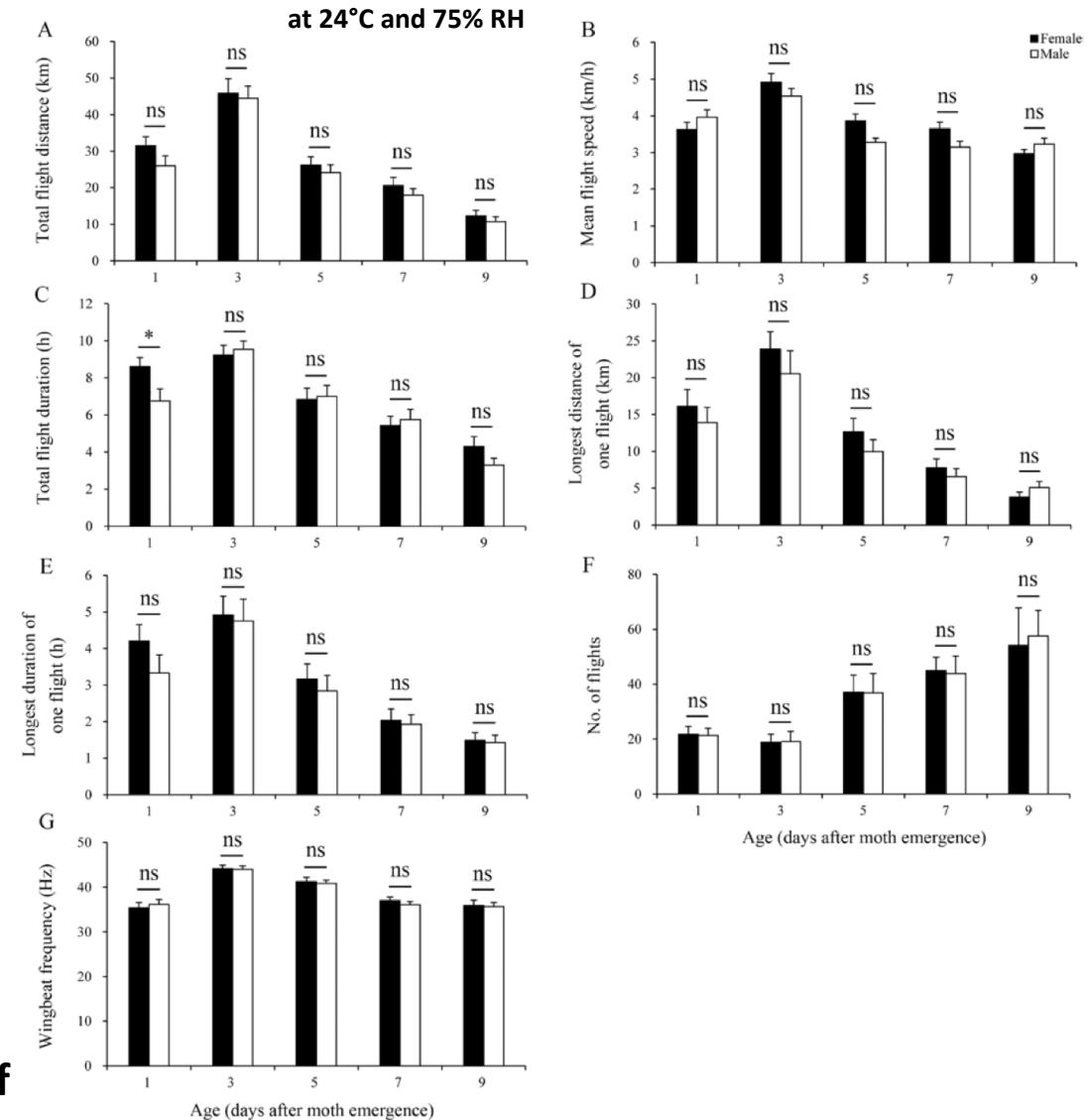
First test the age effect. Have multiple age groups (1,11,21,31,41,51,61 days since wing formation). Similar set-up: One mother (minimize genetic variability).



**~2 month lag to get a range of ages**



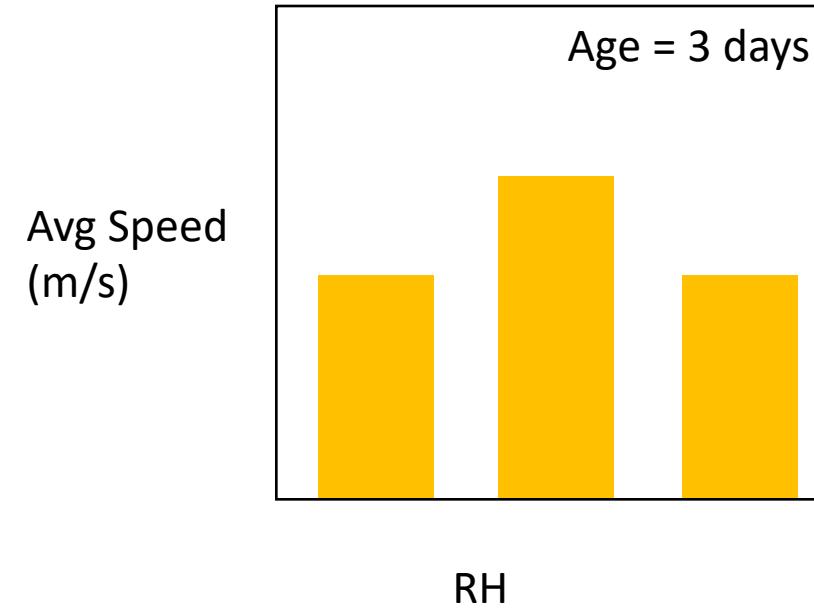
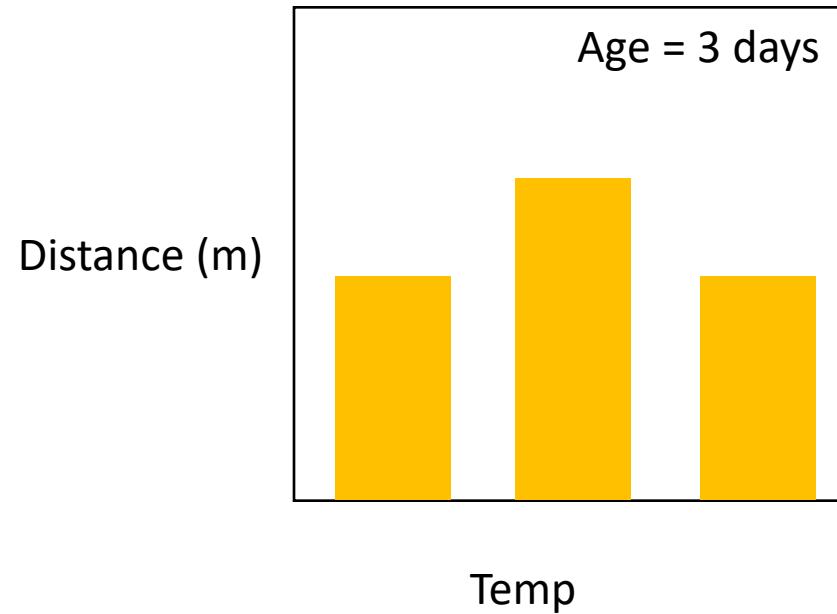
**~7 days of testing**



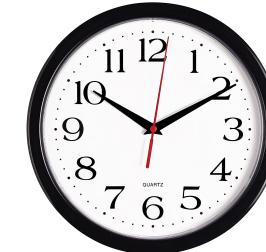
# Age-Controlled Trials

Then, test temperature and RH effect using the age group with the strongest flyers.

Temperature treatments at 12, 20, 28, 36, 44 C (set at 70% RH). Humidity treatments at 40, 55, 70, 85, 100% (set at 28 C). 14:10 (L:D). 8 test days total if can test all bugs in one day and using only age = \_\_\_\_\_ bugs.



.....etc.



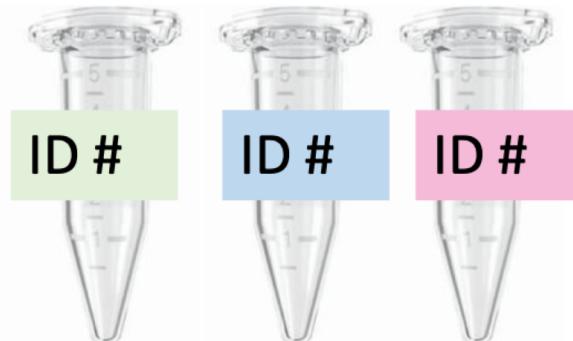
~9 days

# Age-Controlled Trial Methods

## 1. 2 Months of Raising Bugs

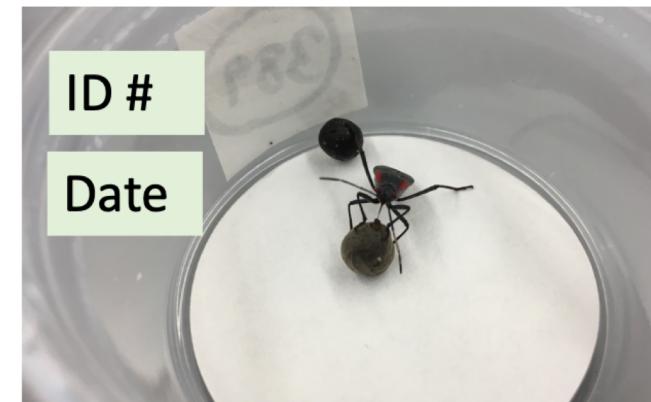
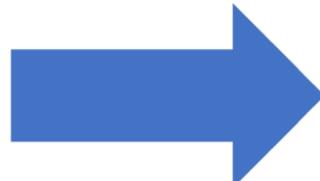
### 1. Collect eggs during the standardized flight trials.

1. Place egg in 2ml tube while counting eggs laid by female. Label tube and fill out datasheet:



	A	B	C	D	E	F	G	H	I	J	K
1	gen1_ID	day_hatched	population	viable?	mother_ID		gen1_ID	day_hatched	population	viable?	mother_ID
2											
3											
4											

2. Store eggs in incubator (same settings as mother). Place eggs that hatch into bug home and throw away inviable eggs. Fill out datasheet and label ID and age of egg on bug home.



2 months from  
October 14

# Age-Controlled Trial Methods

2. Day before trial (Age Assay):
  - I. Test up to 60 field bugs per treatment. Select equal number of males and females, half mainland and half keys, randomly.
  - II. Generate datasheet(s) the day before.
  - III. Paint bugs.
  - IV. Give Gatorade picks.
3. Testing Day (Age Assay):
  - I. Set incubator to treatment of 28 T and 70% RH.
  - II. Record mass and which females laid eggs.
  - III. Test up to 60 bugs in one day for that treatment. Follow the same experimental set-up used in standard flight trials.
  - IV. Count how many eggs a tested female laid.
  - V. End flight testing at 4 PM each day
4. Repeat step 3 until all age groups accounted for



~2month lag? +  
7 days

# Age-Controlled Trial Methods

5. Day before trial (Temperature and RH Assay)
  - I. Select age group of the strongest flying bugs.
  - II. Test up to 60 field bugs per treatment. Select equal number of males and females, half mainland and half keys, randomly.
  - III. Generate datasheet(s) the day before.
  - IV. Paint bugs.
  - V. Give Gatorade picks.
6. Testing Day (Temperature and RH Assay)
  - I. Set incubator to treatment (e.g. 28 T and 70% RH).
  - II. Record mass and which females laid eggs.
  - III. Test up to 60 bugs in one day for that treatment. Follow the same experimental set-up used in standard flight trials.
  - IV. Count how many eggs a tested female laid.
  - V. End flight testing at 4 PM each day
7. Repeat steps 5-6 for a new treatment (8 treatments total)

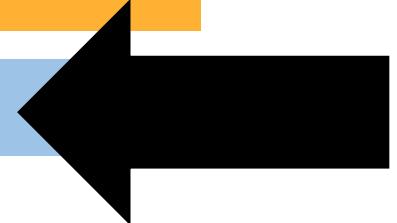


**9 days to  
January 7**

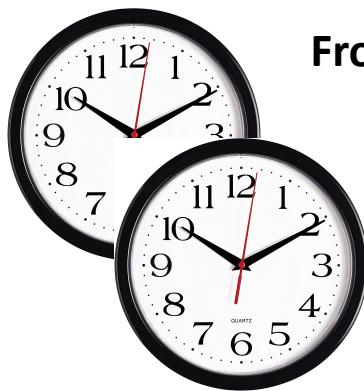
# Two Main Test Groups

1. Testing Field Bugs (Preliminary Extreme Trials)
2. Testing 1 Generation Down of the Field Bugs (Age-Controlled Extreme Trials)

3. Timeline & Materials



# Total Time



From September 22 (end of standard flight trials)

8-16 days

To October 14 (end of extreme preliminary trials)

2 months + 2 month lag?



To December 14 (end of bug raising)

Need ~17 days\*



To Jan 8 (end of age-controlled trials)

\*Runs through holidays

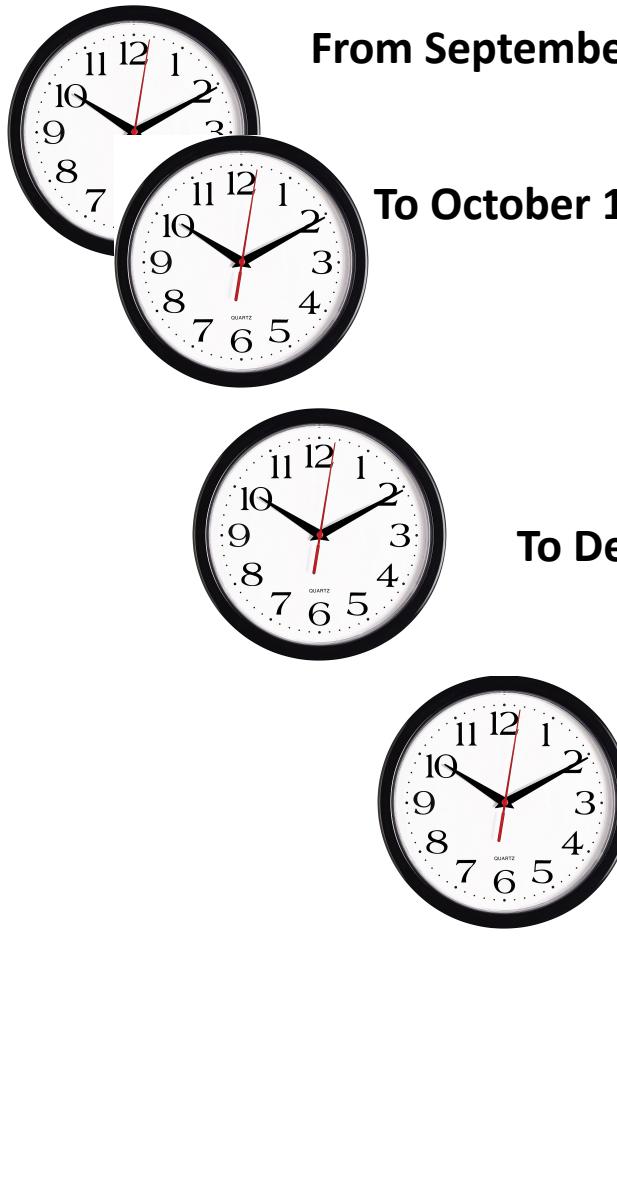


3.75 months

End of April (paper submitted)

Not enough time

# Total Time



From September 22 (end of standard flight trials)

8-16 days

To October 14 (end of extreme preliminary trials)

2 months + 2 month  
lag?

To December 14 (end of bug raising)

Need ~17 days\*

To Jan 7 (end of age-controlled trials)

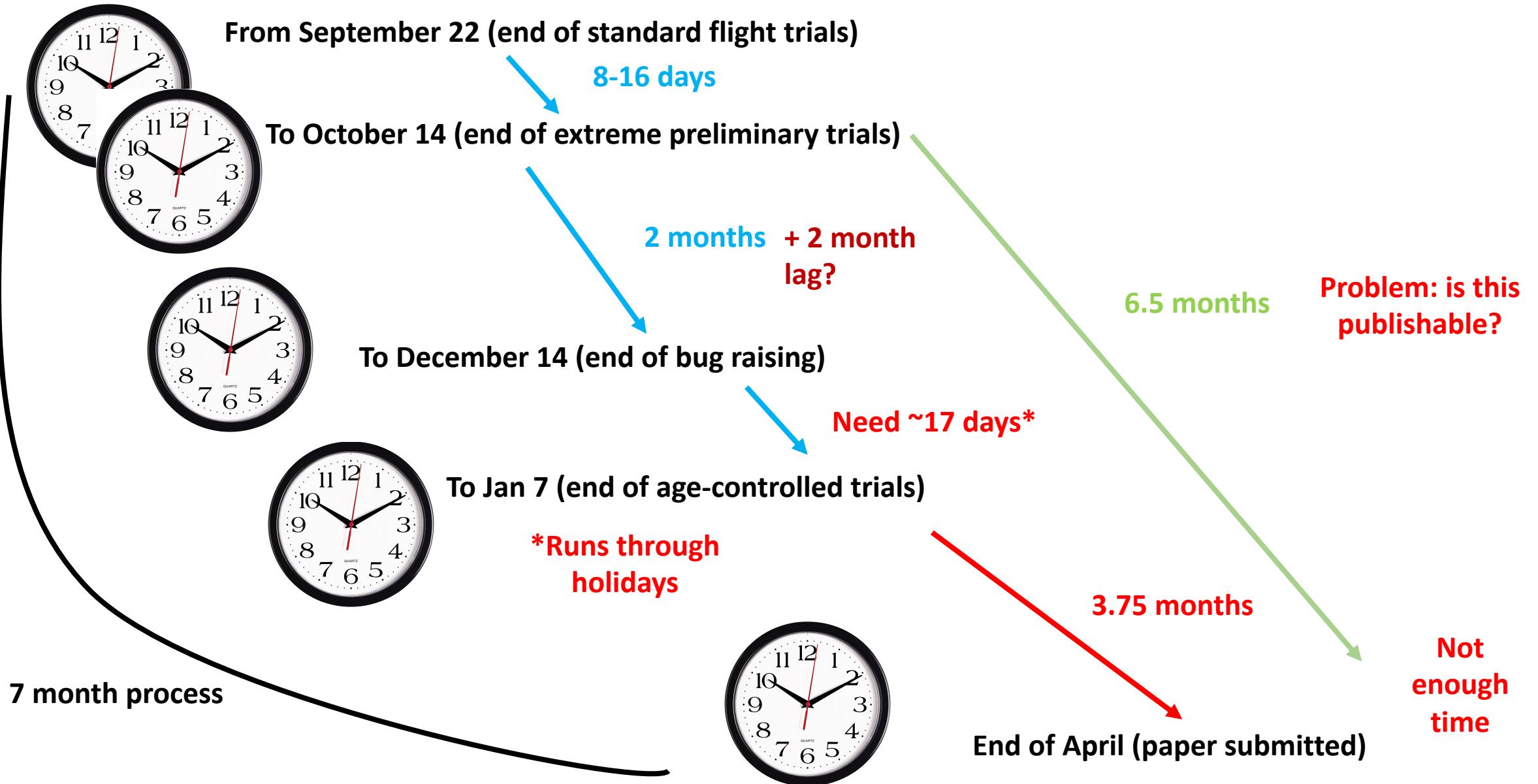
\*Runs through  
holidays

6.5 months

3.75 months

Not  
enough  
time

# Total Time



# Materials – datasheet 1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	ID	died?	chamber	set_number	test_date	time_start	time_end	population	flew	flight_type	ID	mass	temp	RH	NOTES
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															

- Usual flight trial datasheet

# Materials – datasheet 2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	ID	date	num_eggs	population		ID	date	num_eggs	population		ID	date	num_eggs	population
2														
3														
4														
5														
6														
7														
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31														
32														
33														

- Usual flight trial egg datasheet

# Materials – datasheet 3

	A	B	C	D	E	F	G	H	I	J	K
1	gen1_ID	day_hatched	population	viable?	mother_ID		gen1_ID	day_hatched	population	viable?	mother_ID
2											
3											
4											
5											
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32											
33											

For age-controlled trials:

- Can group by family
- Keep track of age
- Keep track of viable/nonviable eggs – something missed during the last trials

# Materials

- Datasheets
- Bug homes (filter paper, seeds, 2 mL tubes, cotton, Gatorade)
- 2ml tubes
- Tube racks
- Incubator Space
- Metal paint + toothpick