Mitochondrial abundance and organism performance

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Background

- **Mitochondria** are the cellular structures that produce **energy** (releasing it from the **nutrients** by consuming **oxygen**, O_2)
- **Whole animal O_2 consumption** rate provide information about basal **energy** requirements (the so-called minimal cost of living, i.e., resting metabolic rate, RMR)
- **Mitochondrial** abundance is expected to influence metabolic rate and hence **whole organism oxygen consumption**
- **Mitochondrial** abundance might change with age and contribute to **senescence**
- RMR is expected to **influence** animals' performance, i.e., the capacity to allocate energy to other costly processes such as **flying**

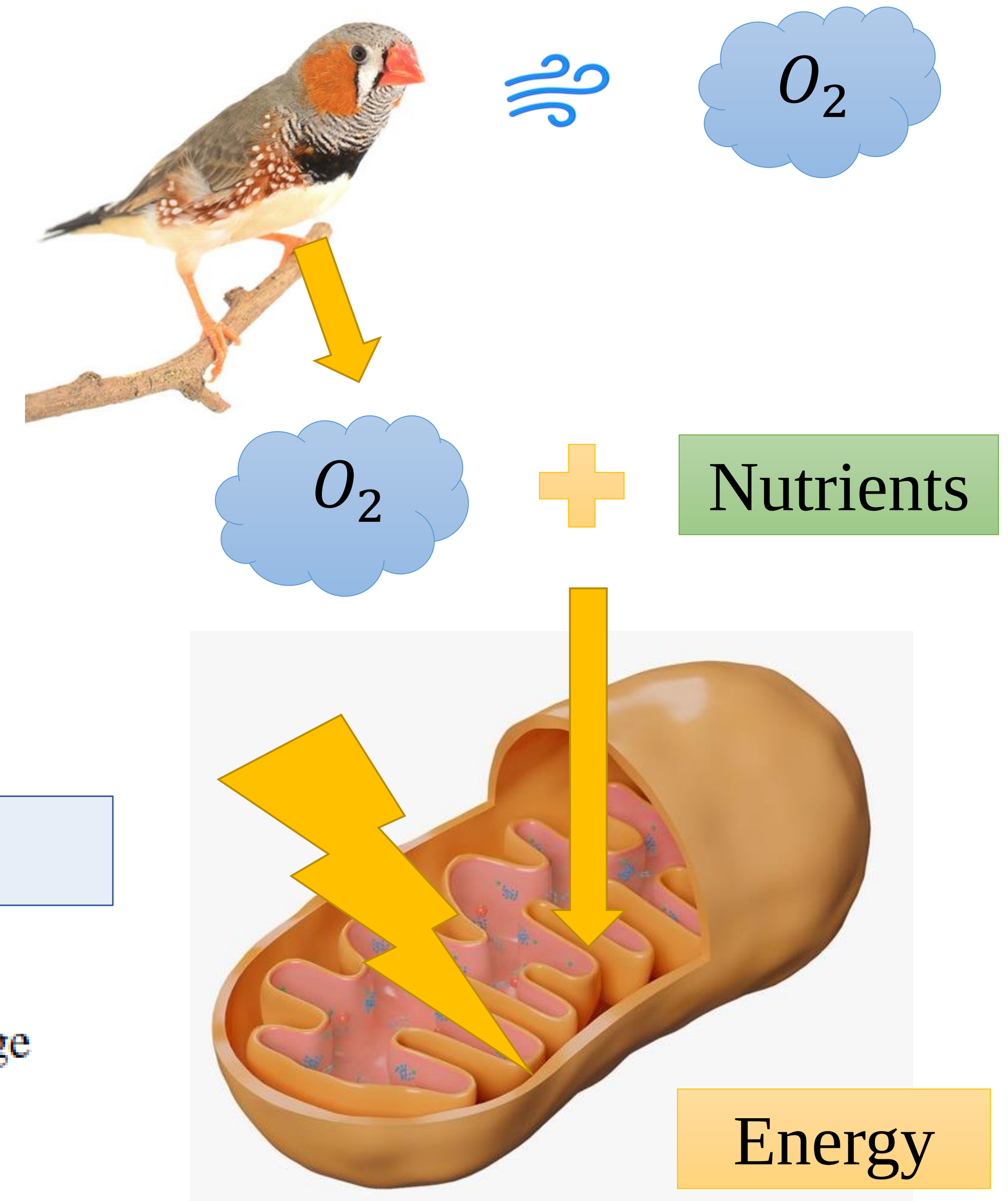

Figure 1: Schematic representation of the relationship between whole animal oxygen consumption and energy generation in the mitochondrion at the cellular level.

Questions addressed in this project

1: Does mitochondrial abundance change with age?

a) Changes in mitochondrial abundance after hatching seem to be tissue specific (increase in muscle and decrease in liver of broilers) (Figure 2)

b) In adults, a reduction in the number of mitochondria has been suggested as a potential hallmark of aging in many species, including birds (Figure 3)

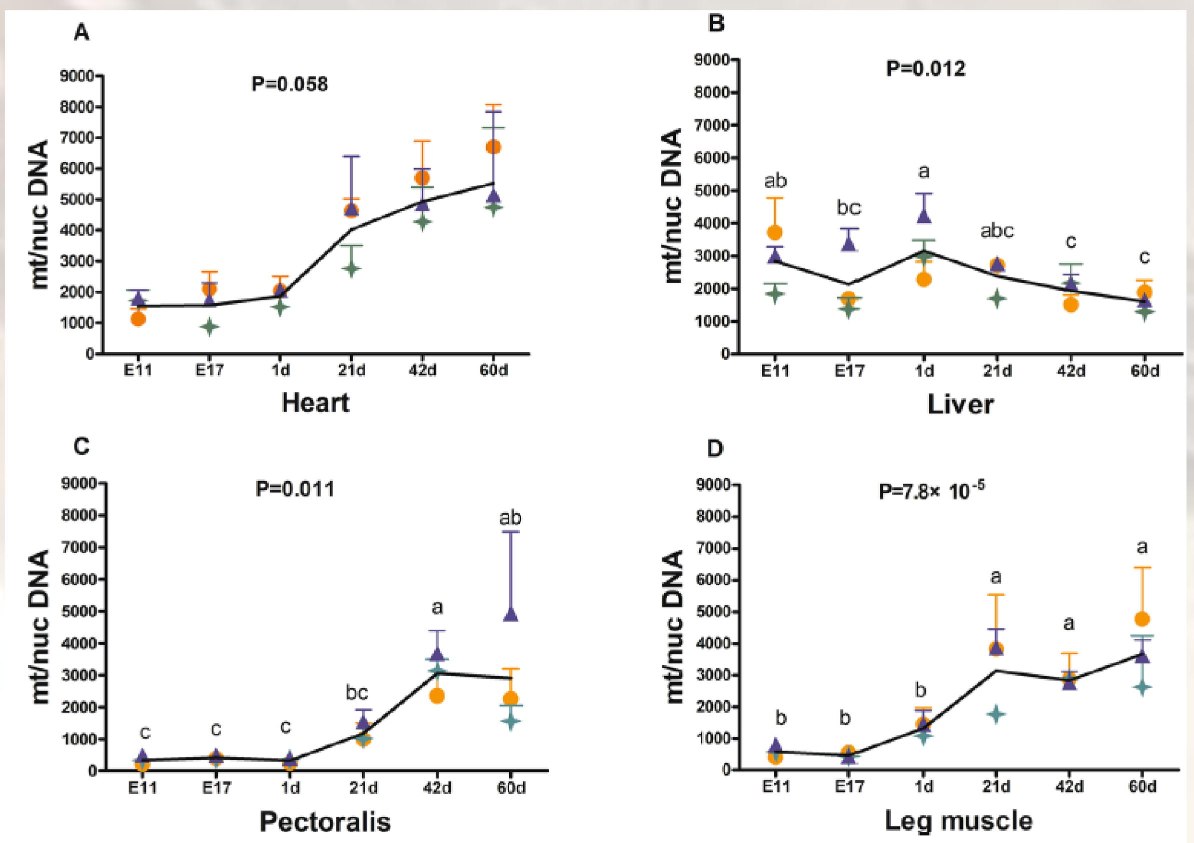

Figure 2: Changes in mitochondrial abundance with age in the heart, liver, pectoralis and leg muscle of broilers (adapted from Zhang *et al.*, 2020)

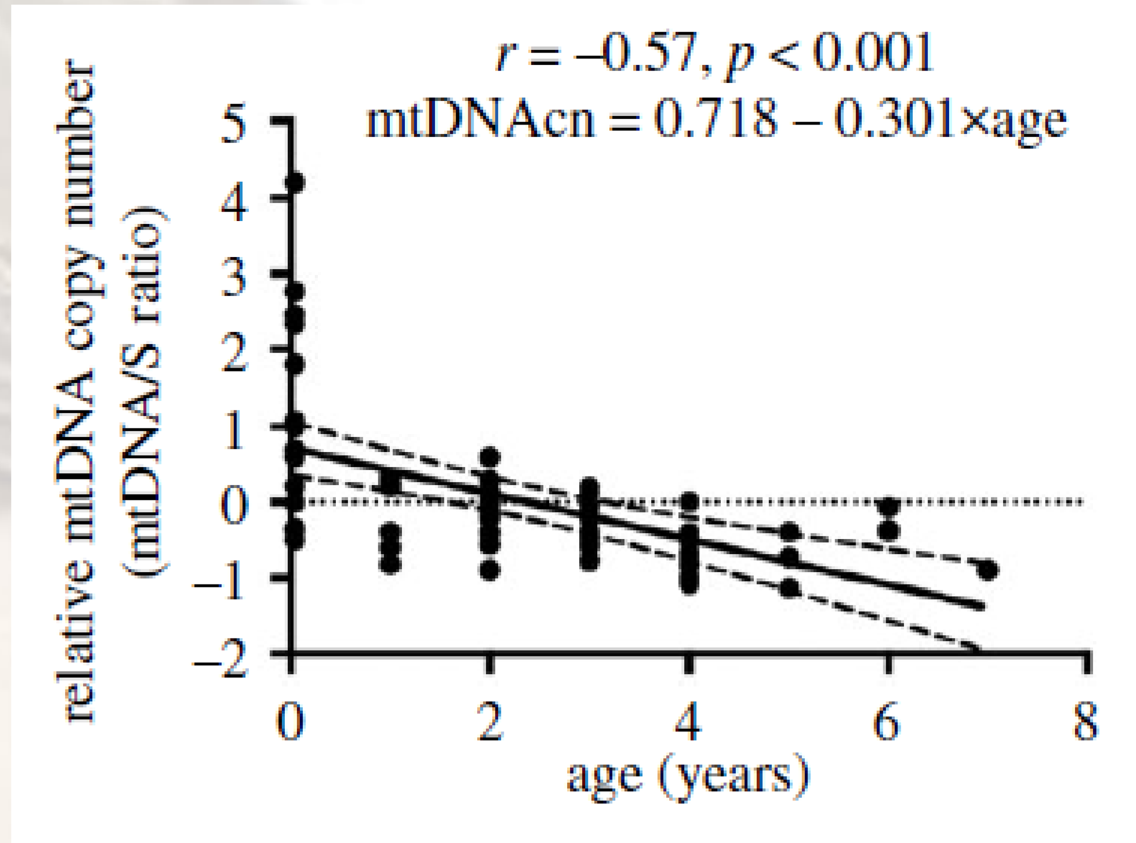

Figure 3: Decline in mitochondrial abundance with age in red blood cells in a wild population of collared flycatchers (adapted from Stier *et al.*, 2020)

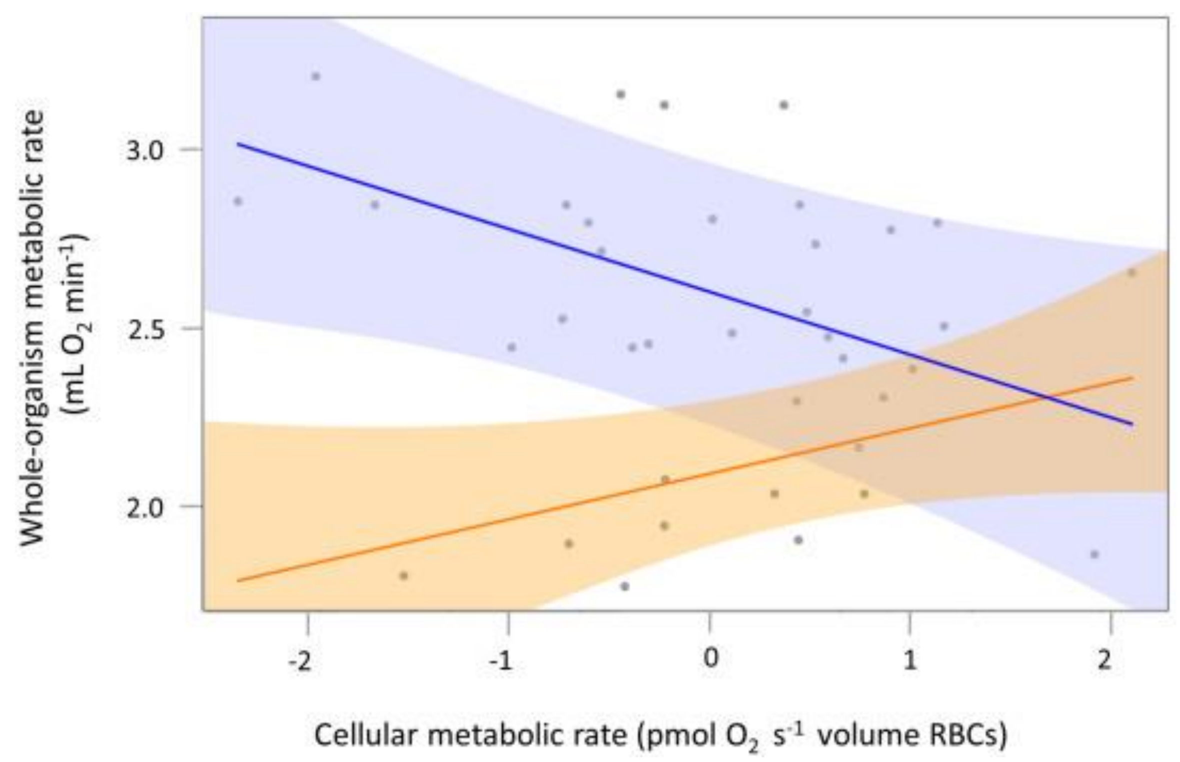

Figure 4: Positive correlation (orange trend) between cellular metabolic rate in red blood cells and whole organism metabolic rate in captive great tits (adapted from Malkoc *et al.*, 2021)

2: Does mitochondrial abundance in red blood cells (RBCs) predict whole animal baseline energy consumption (RMR)?

- In non-stressed birds, cellular metabolic rate in red blood cells and whole organism metabolic rate seem to be positively correlated (Figure 4)

3: Do differences in RMR influence flying performance?

- Higher RMR could be associated with a lower aerobic scope, i.e. birds would have less capacity to increase their energy expenditure for costly activities

Methods

- I used stored RBCs samples collected from (n = 24) captive male zebra finches (*Taeniopygia guttata)* as part of an ongoing longitudinal study

1. DNA was extracted using a Puregene **DNA extraction kit** (Qiagen) (Figure 5)

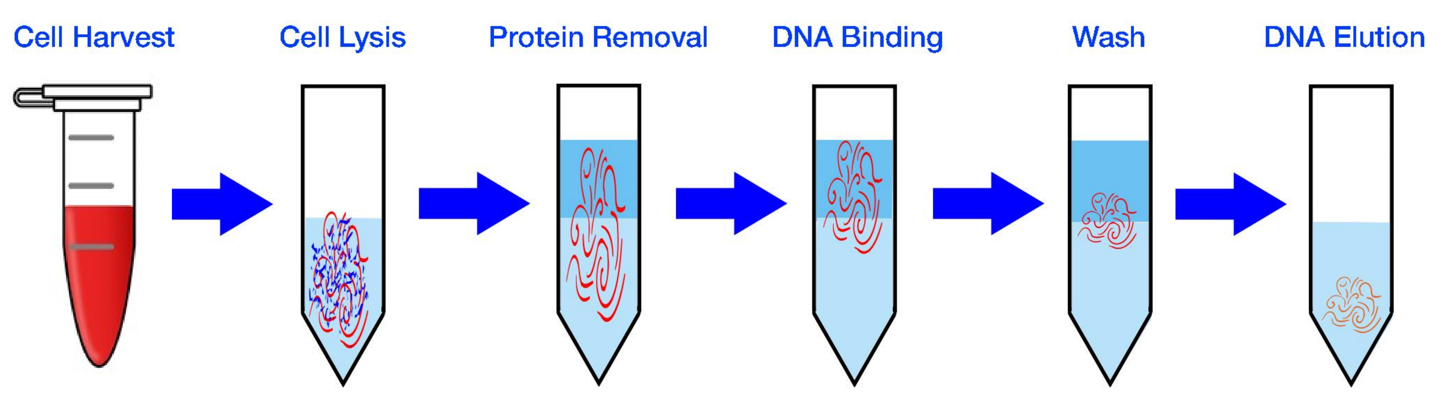

Figure 5: Stepwise diagram of a DNA extraction protocol

3. RMR was quantified using **flow-through respirometry** (Criscuolo *et al*., 2008) (Figure 6) to measure the rate of oxygen consumption while birds were within their thermo-neutral zone, following overnight fast and in darkness to ensure they remained inactive

Figure 6: Schematic diagram of the principle and experimental setup of a flow-through respirometry system. Oxygen is measured before entering the chamber where the bird is kept and after exiting it, allowing estimation of whole animal oxygen consumption rate

2. Mitochondrial abundance was estimated using **quantitative polymerase chain reaction (qPCR)**, to calculate the relative ratio of mitochondrial genomes (quantified by the presence of the mitochondrial gene ND2 as in Knief *et al*., 2021) to nuclear genomes (identified by the number of copies of the nuclear gene RAG1)

4. Flying performance (wing beat frequency during the linear phase of flight) was estimated from **videos of adult birds** flying upwards in a controlled experimental setting (Figure 7).

$$\text{Wing beat frequency} = \frac{\text{number of beats}}{L_L\ distance\ (\text{cm}) \cdot time(s)}$$

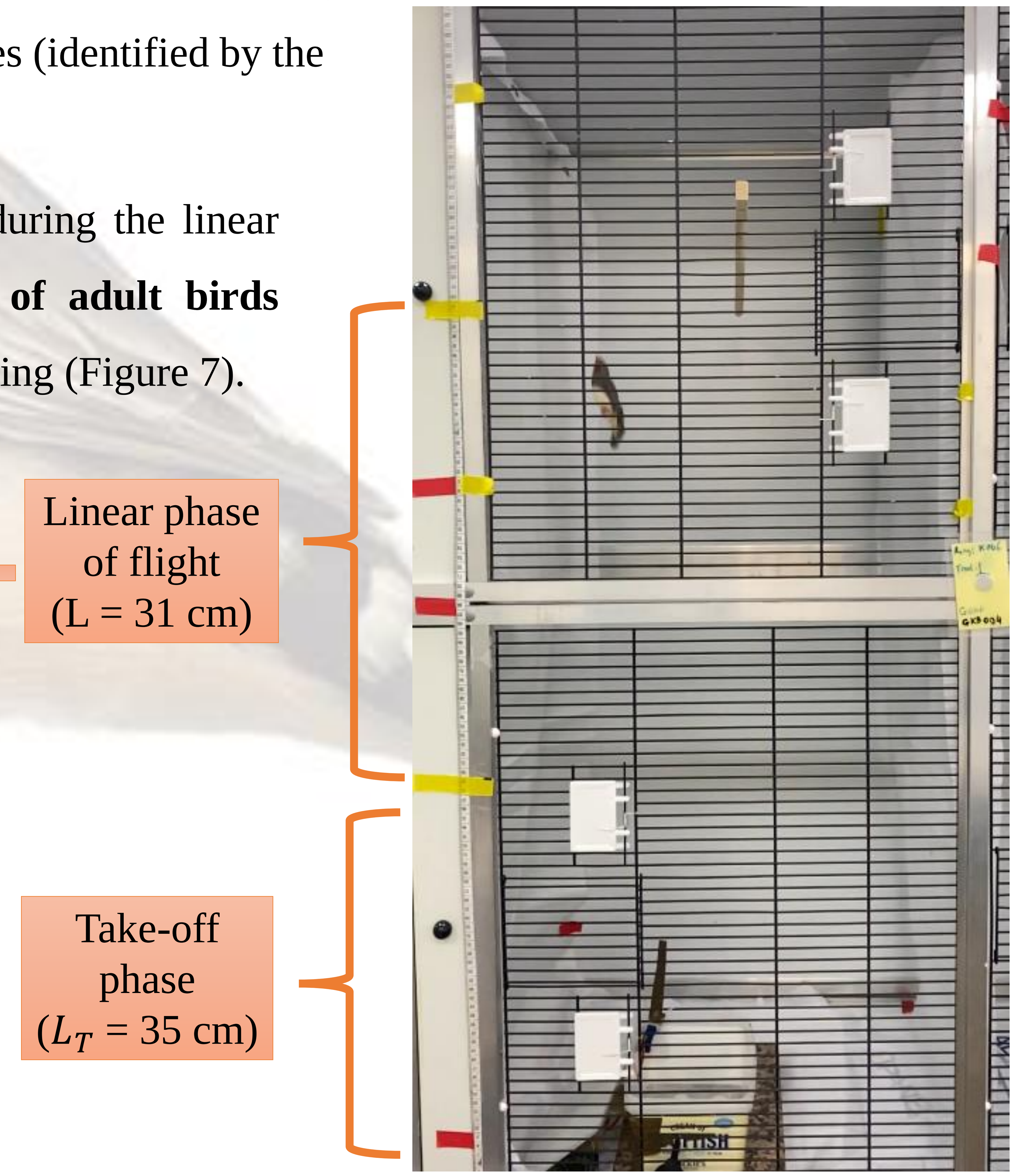

Figure 7: Experimental set up for flight performance test (designed by Ivimey-Cook)

Results and Discussion

1. Steep decline in mitochondrial abundance during the juvenile stage but no changes throughout adulthood (n = 24) (Figure 8)

- Chicks might **compensate** for their **reduced RBC number** (lower haematocrit) by having **more mitochondria** per RBC
- **Haematocrit** tends to **decline with aging in adults** (Coughlan *et al*., 2022; Brown *et al*., 2020), which suggests that adult's capacity to generate energy might decline with age (same number of mitochondria per red blood cell but fewer red blood cells; hence, fewer mitochondria overall)
- Thus, we found that **mitochondrial abundance**, at least in RBCs, could **contribute to senescence**

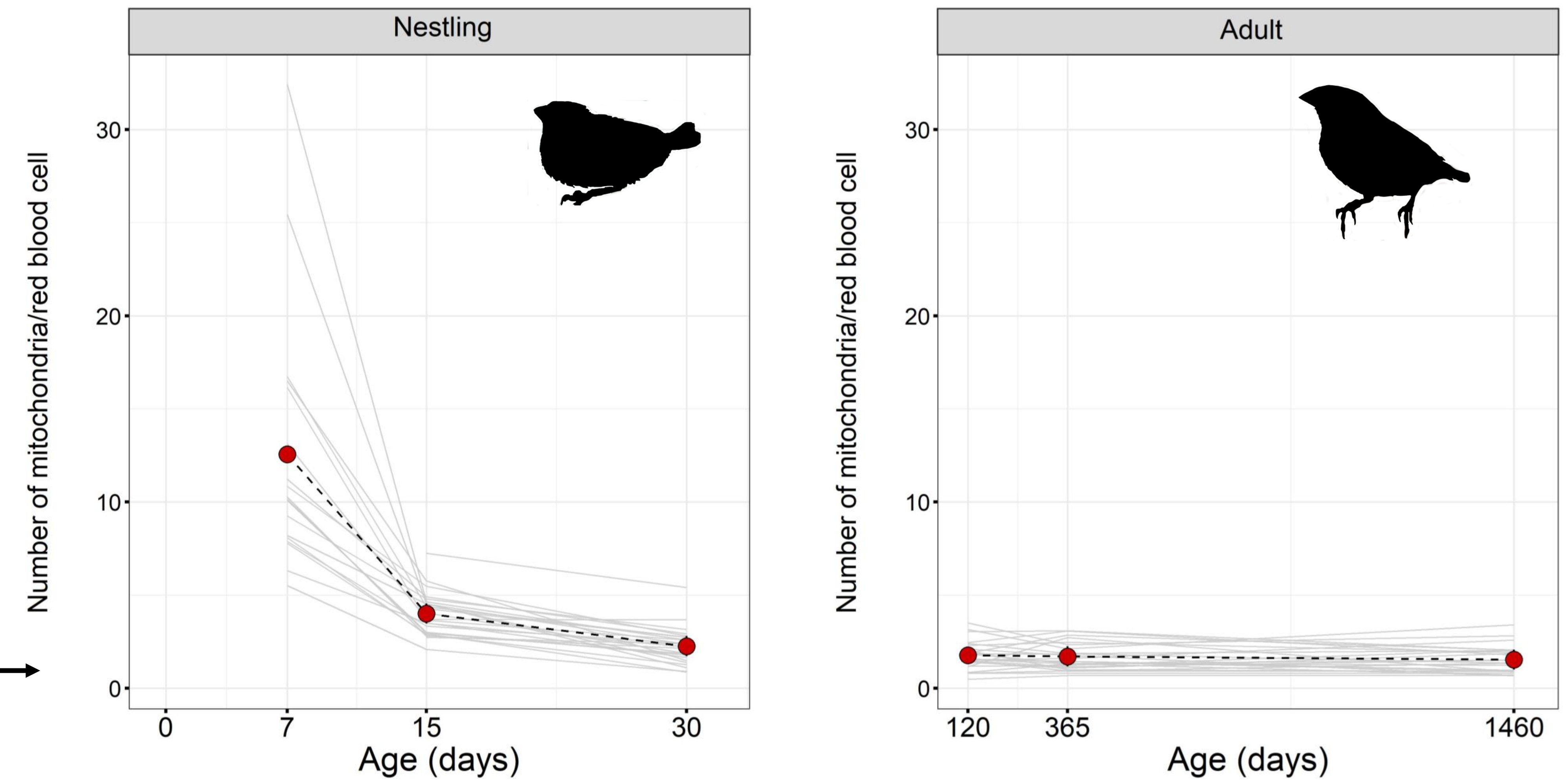

Figure 8: Longitudinal changes in mitochondrial abundance with age in red blood cells of captive male zebra finches

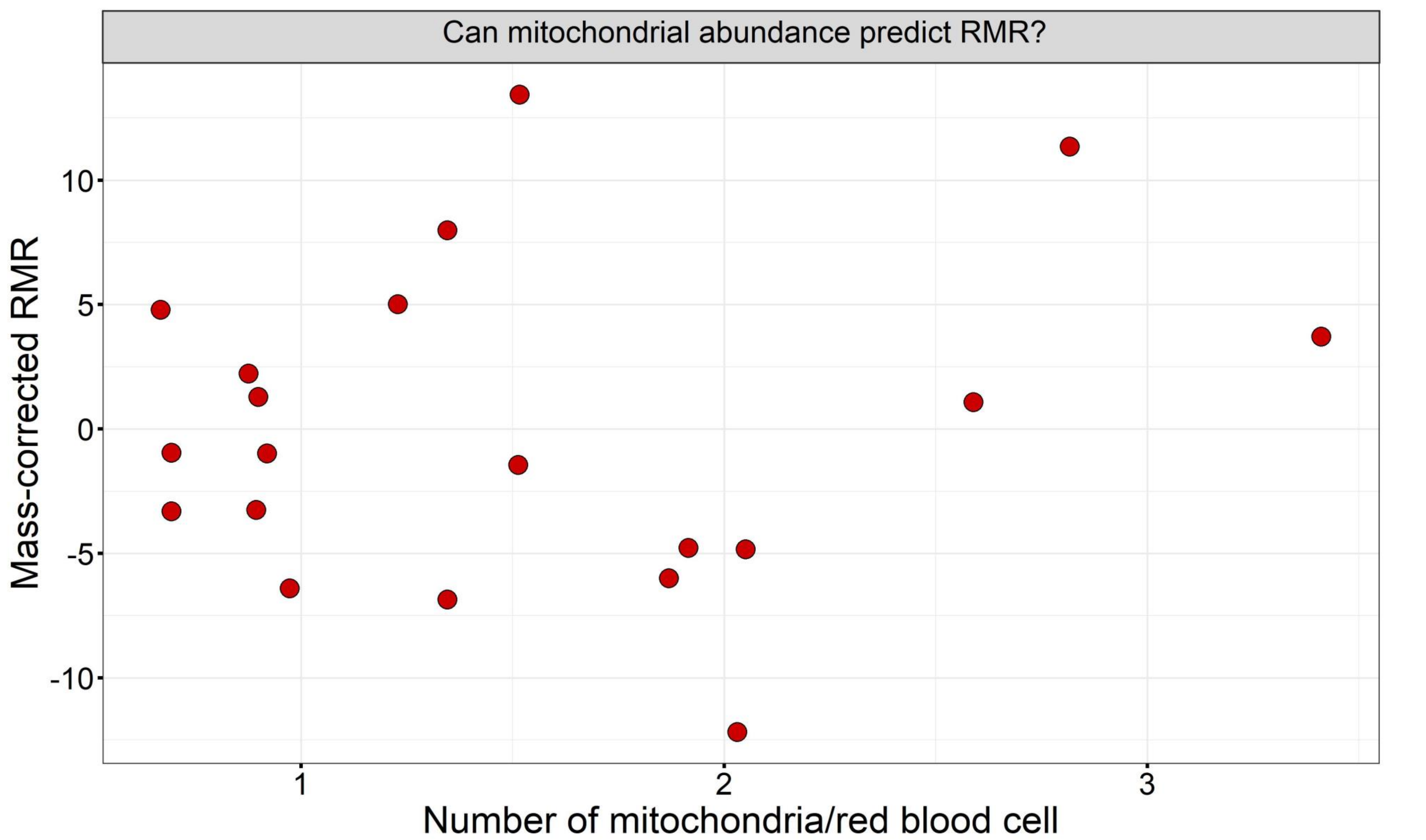

Figure 9: Relationship between mitochondrial abundance in red blood cells and whole organism metabolic rate (corrected by body mass) in 4-year old adult male zebra finches

2. No association between RBC mitochondrial abundance and whole animal energy consumption ($p > 0.05$; n = 20) (Figure 9)

- The **role of mitochondria in avian RBCs remains unclear** (Stier *et al*., 2013), which might explain the lack of an obvious link between energetic requirements in RBCs and at the whole organism level
- Other tissues with a higher contribution to overall energy requirements (liver, skeletal muscle) might show a relationship

3. Positive correlation between RMR and flying performance ($p < 0.01$; n=20) (Figure 10)

- Individuals with a **higher baseline cost of living** need to **flap faster** to cover the same distance.
- Birds with **high RMR** might need to **spend more energy to carry out other daily activities**, with potential consequences for survival under challenging environmental conditions.
- If a higher RMR was correlated with a lower aerobic scope, individuals with higher metabolic rates could face a higher risk of predation under natural conditions (Buttemer *et al*., 2019)

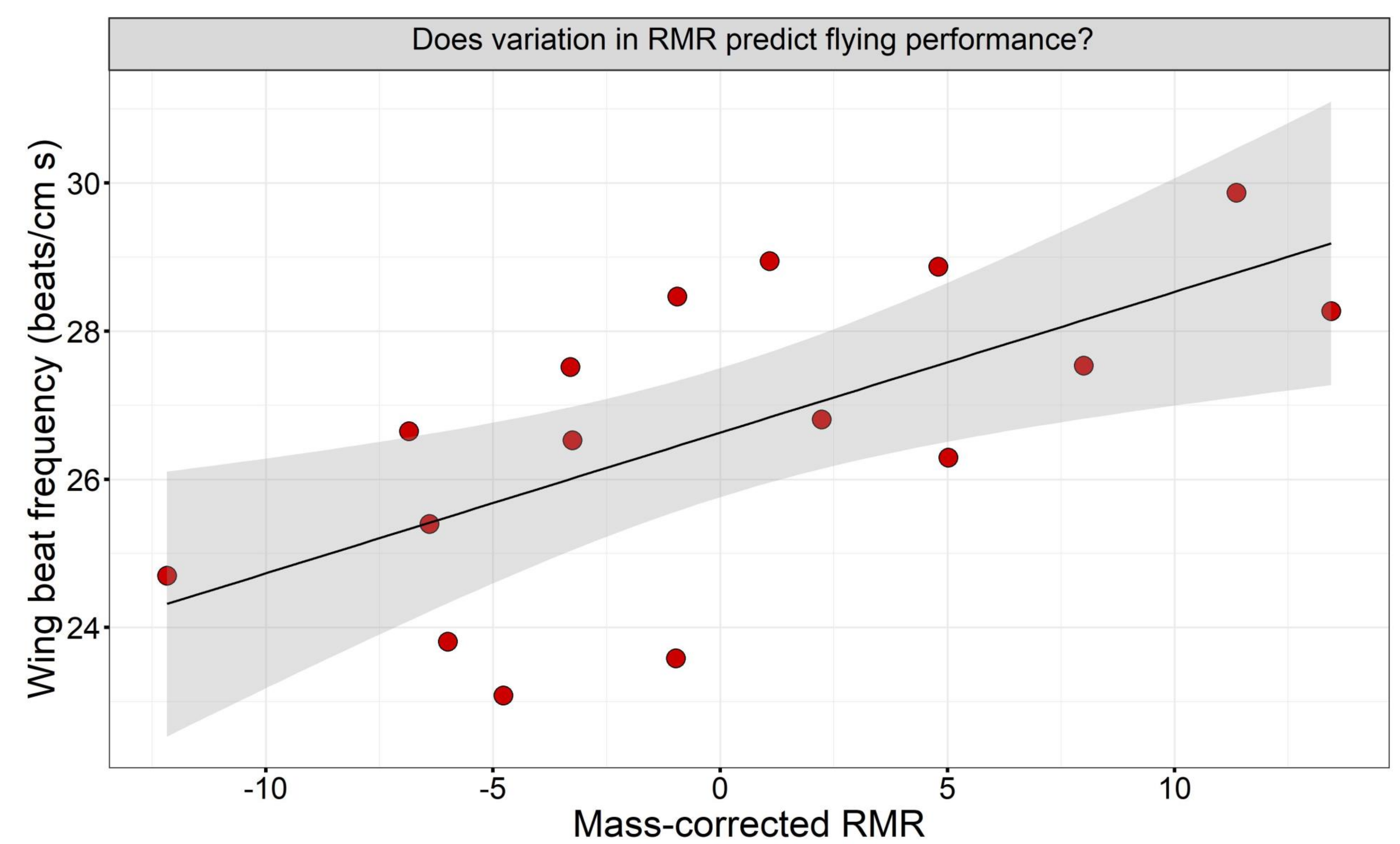

Figure 10: Positive correlation between whole organism metabolic rate (corrected by body mass) and flying performance (wing beat frequency) in 4-year old adult male zebra finches

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