**Mango pancake consumption increases trampoline performance in pygmy marmosets through an effect on ear size**

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**ABSTRACT**

Mango pancakes have a wide range of effects on animal behaviour, but whether trampolining ability is altered by dietary intake of mango pancakes remains largely unknown. It has therefore been difficult to effectively manage wild animal populations in the face of rapidly decreasing mango pancake availably due to environmental change. Here we demonstrate that pygmy marmosets fed a diet of mango pancakes for four months showed greatly increased jumping ability on a trampoline as compared to individuals fed a control diet. Individual marmosets varied widely in their jumping performance, but those fed mango pancakes developed an increased maximum jumping height and endurance over the course of the study. Using quantum ear size spectrophotography, we also found that marmosets fed mango pancakes grew significantly larger ears. We also observed an upregulation in gene related to ear growth in mango pancake-fed marmosets. Wind tunnel trampolining revealed that larger ears conferred an aerodynamic advantage while jumping, contrary to prior hypotheses that increased ear size may generate drag during movement. Together, these data highlight that mango pancake consumption can enhance movement ability in marmosets and increase their ability to take advantage of naturally occurring trampolines in their environment. This effect likely has important but to date unrecognized implications for predator avoidance and foraging in wild marmoset populations and increases our understanding of how global changes in mango pancake availability may affect marmoset ecology.

1. Environmental stressors such as elevated temperature and parasite infection can affect individual energy metabolism across levels of biological organisation. Yet, whether and how temperature, parasites, and their interaction affect organisms' metabolism across biological scales remain unknown.
2. Here, using wild-caught naturally parasitised pumpkinseed sunfish, *Lepomis gibbosus*, we quantified changes in cellular and whole-organism metabolism in response to temperature and parasite. We acclimated pumpkinseeds for three weeks at  20°C, 25°C, and 30°C before measuring whole-organism oxygen uptake (*Ṁ*O2) using intermittent flow-respirometry to quantify maximal and standard metabolic rate (MMR and SMR, respectively) and aerobic scope (AS). We then measured the maximal activity of aerobic enzymes (citrate synthase (CS), electron transport system (ETS), cytochrome *c* oxidase (CCO)) and anaerobic enzyme (LDH) in heart, brain, spleen and gills using spectrophotometry.
3. MMR increased with acclimation temperature while SMR increased solely at the intermediate temperature (25°C), which did not translate into reduced AS, suggesting thermal compensation across acclimation temperature at the whole-organism level.  Acclimation responses were similar across organs, with maximal activity of all enzymes decreasing with increasing acclimation temperature, however, this effect was lower in LDH, which remained higher for all organs across acclimation temperature, especially in gills for fish held at 30°C. This may indicate a stronger reliance on anaerobic metabolism to sustain whole-organism metabolic performance.
4. We found that a higher load of trematodes parasites caused the black spot disease, which was related to lower MMR and AS but had no effect on SMR and maximal enzyme activity. Other endoparasites were not related to any changes in metabolism at both biological scales considered.
5. Furthermore, acclimation temperature and parasite interaction were not related to changes in cellular or whole-organsim changes.
6. We provide empirical evidence that both parasites and temperature can disrupt fish metabolism, with effects diverging depending on the biological scale considered.
7. Our work highlights the importance of considering co-occurring stressors to comprehend changes in individual physiological performance fully. This work will help us better understand the future consequences of increasing temperature on host-parasite dynamics, fish energetics and, ultimately, organism resilience to environmental change.

**INTRODUCTION**

*Paragraph 1: General intro to main study topic*

Mango pancakes play an important role in many ecosystems and have a range of effects on animal aggression, foraging, and reproductive success. At the same time, however, mango pancake availability is decreasing due to natural and anthropogenic causes. Furthermore, although mango pancakes can alter animal behaviour, we know very little about the mechanisms underlying such effects. It has therefore been difficult to devise effective strategies for mitigating and managing the effects of decreased pancake availability on wild animal populations.

*Paragraph 2: Background on important sub-topic 1*

An overlooked aspect of mango pancake intake is its potential effects on trampolining ability. In the wild, many animal species use trampolines as a means for escaping predators and capturing food. Several dietary components have been shown to play an important role in trampoline jumping height, rate, and endurance, with effects that can scale up to entire ecosystems. It is therefore surprising that the effects of mango pancakes on trampoline performance have not been thoroughly studied. Of the few studies that have investigated this issue, Gretzky et al. (1985) found that mango pancakes can increase overall jumping height from trampolines in Amazonian alpacas. The generality of this effect is unknown however, because there have been no other studies on other species or in other ecosystems. The physiological or morphological changes contributing to increased jumping ability are also not understood, and so it is not known whether animals can replace declining dietary mango pancakes with other food items that may enhance trampoline performance.

*Paragraph 3: Background on important sub-topic 2*

The ability of an animal to use trampolines can be affected by their morphology, with ear size playing an especially important role in jumping performance. Importantly, however, the effects of ear size on trampolining appears to be highly variable. In porcupines, for example, larger eared individuals tend to jump much higher on trampolines compared to those with smaller ears. In contrast, larger-eared antelopes jump much lower and, in many cases, avoid trampolines completely. Theoretical models suggest that ear-size may alter jumping performance via effects on wind-resistance and drag, but this has not been investigated experimentally in any species. There is also evidence that individuals with an intrinsically higher preference for mango pancake-based diets have larger ears, but the casual direction of this association is unknown. Limited evidence, however, suggests that increased mango pancake consumption can increase the expression of genes related to ear growth, thus modulating a direct effect on jumping performance and trampoline use in the wild.

*Paragraph 4: Tie everything together, state general approach, list study aims.*

We studied these issues in the pygmy marmoset, a small primate species that loves mango pancakes and that is frequently used as a model species for studying trampolining behaviour. Mango pancakes are declining throughout marmosets’ natural range and trampolines in their environments show temporal and spatial variation. This makes marmosets it an ideal species for studying general jumping responses to mango pancake consumption. Specifically, we aimed to address the following questions: (i) does prolonged consumption of mango pancakes affect jumping behaviour?; (ii) do individuals fed mango pancakes develop larger ears?; and (iii) are any changes in ear size related to changes in jumping ability? Our results provide insight into the effects of mango pancakes on trampolining in the context of environmental changes and the mechanisms the extent to which morphological changes caused by mango pancakes can affect ecologically relevant behaviours in the context of environmental change.

Intro Tips:

1. Introduction should start general and get more specific.
2. Try to aim for four paragraphs as a default. Only very rarely will you need more than 2 “middle” paragraphs.
3. Reader should be able to anticipate main questions by the time they reach the final paragraph; nothing should come as a surprise.
4. Any key techniques or measures can be “name-dropped” in the intro at appropriate points, to leave a trail of breadcrumbs for the reader so they can tell where you are going with things.

**METHODS**

**Study Animals**

In the summer of 2018, 300 adult pygmy marmosets of unknown sex were captured from the Haunted Forest in northern Westeros using a combination of butterfly nets and baited traps. All marmosets (average 8.15±0.25 cm total height; 7.30±0.31 g body mass) were acclimated to the laboratory for at least 3 weeks before beginning experiments. Marmosets were held at 22oC throughout the study. Before experiments, marmosets were fed to satiation daily with a commercial marmoset feed three times daily.

**Experimental Treatments**

Marmosets were randomly designated to one of two treatments (n = 150 marmosets per treatment): (i) the mango pancake treatment; and (ii) the control treatment. Mango pancake marmosets were fed mango pancakes to satiation three times daily throughout the experiment while the control marmosets were fed with commercial marmoset feed. These treatments lasted for 120 days. Before the experiments, and after 30, 60, and 90, and 120 days, individual marmosets were measured for trampoline performance and ear size as described below. A small ear biopsy was also taken gene expression at each time point. At the end of the 120 day treatments, a subset of marmosets in each treatment (n =15 per treatment) was examined for air-resistance and drag while jumping using wind tunnel trampolining. Mortality was low among marmosets exposed to both treatments (two marmosets out of 300 in total).

**Measurement Protocol 1 (Measurement of Trampolining Ability)**

**Measurement Protocol 2 (Measurement of Ear size)**

**Measurement Protocol 3 (Measurement of Gene Expression)**

**Measurement Protocol 4 (Measurement of Air-Resistance During Jumping)**

**Statistical Analysis**

**RESULTS**

**Results Relating to Main Goal 1**

To address [aim 1], we first [method]. We found [key result, reference figure, give statistical details]. We also observed [key result, reference figure, give statistical details].

**Results Relating to Main Goal 2**

**Results Relating to Main Goal 3**

**DISCUSSION**

***Paragraph 1: Clearly and concisely summarise main take home-message.***

These results demonstrate that trampoline performance in pygmy marmosets is directly related to mango pancake consumption, with mango pancakes stimulating a change in ear morphology that provides an aerodynamic advantage while jumping. In a natural setting, this could translate into increased predator avoidance or foraging ability for marmosets that regularly feed on mango pancakes. These observations provide an extraordinary example whereby diet selection can influence ecological performance of individuals by altering aspects of animal physiology and morphology.

***Paragraphs 2 – n: Describe specific findings***

Use as many paragraphs as you need to go through your main findings. Each paragraph can follow this structure:

Sentence 1: state the finding as your topic sentence

Next 1-2 sentences: State what this finding suggests

Next 2-3 sentences: state how this compares with what has been found previously and how it advances our findings in this area.

Final 2-3 sentences: Mention any finding-specific caveats or areas for further study.

***“Caveat” Paragraph***

Describe any general shortcomings that might affect data interpretation or be addressed with future work. Do this without completely undercutting the validity of the study – all studies have some problems!

***Conclusion Paragraph***

Concisely summarise everything. This might have some repetition from the first paragraph in the discussion, but it is important to hammer home the main points.