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INTERMITTENT FASTING

Can skipping breakfast improve weight management?

Page 16

HIDDEN SADNESS?

Recognition of faces and emotions during Covid-19 pandemic

Page 38

SENSE OF SYMMETRY, INTRINSIC OR INSTRUCTED?

We ask the macaques!

Page 58

POLLINATORS IN DECLINE

Is balcony vegetation a potential solution?

Page 78



Methodological workshop in Evolutionary Biology – Grant writing
~~Ochotnica Górna~~ Microsoft Teams, 14th-21st September 2020

Table of Contents

List of participants.....	1
Group photo.....	2
Research topics proposed and chosen by participants.....	3
• Project 1: Is the morning “breaking of fast” the most important meal of the day? Intermittent fasting and its role in weight management	
Photo of team members.....	4
Draft project proposal.....	5
Reviews.....	12
Final project proposal.....	16
• Project 2: Behind the mask. Human abilities to face recognition during the Covid-19 pandemic	
Photo of team members.....	24
Draft project proposal.....	25
Reviews.....	30
Final project proposal.....	38
• Project 3: The characteristics and origin of symmetry detection in rhesus macaques (<i>Macaca mulatta</i>)	
Photo of team members.....	44
Draft project proposal.....	45
Reviews.....	55
Final project proposal.....	58
• Project 4: Is balcony vegetation significant for pollinators?	
Photo of team members.....	64
Draft project proposal.....	65
Reviews.....	72
Final project proposal.....	78
Acknowledgements.....	85

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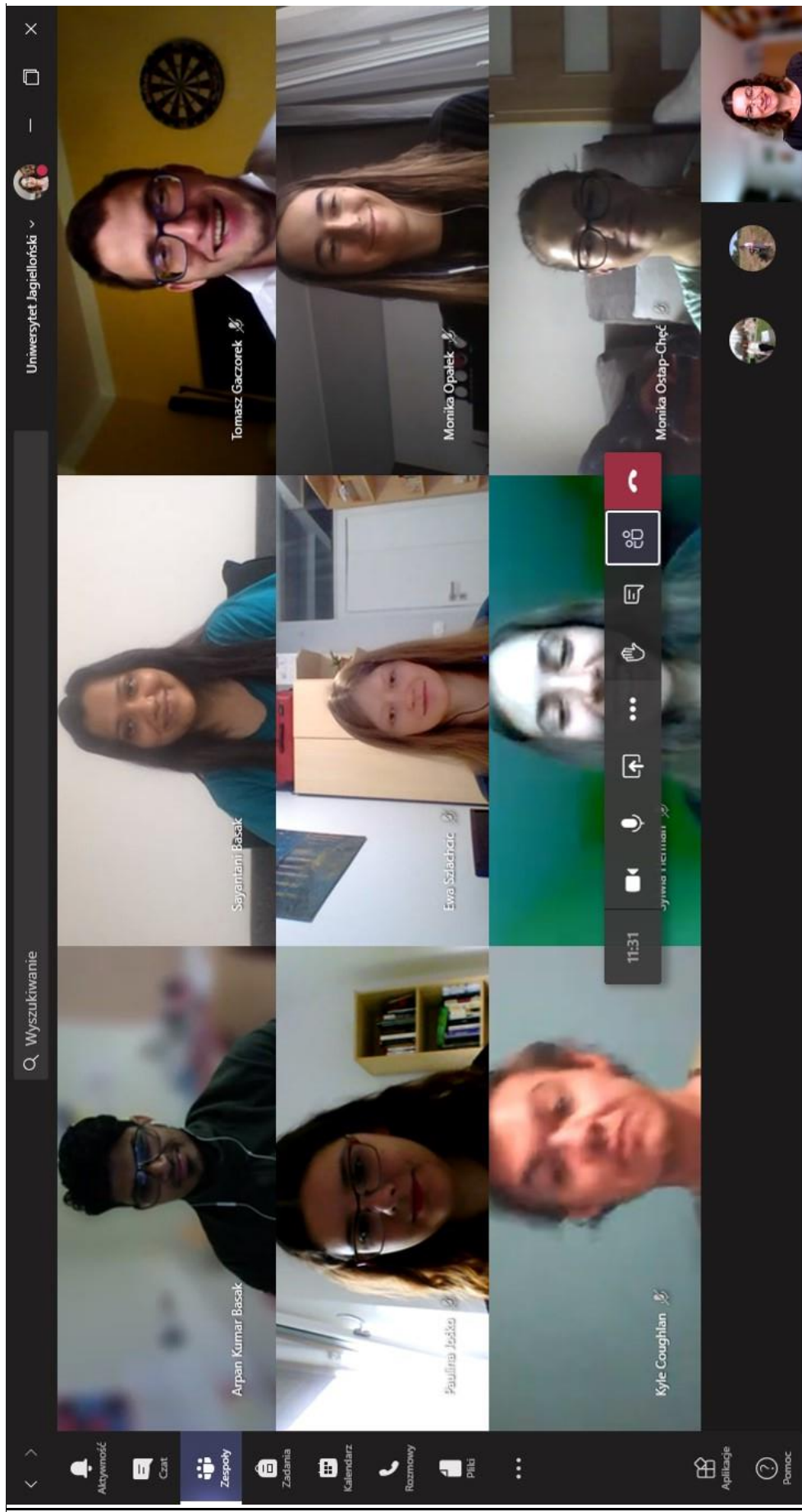
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Group Photo taken on Microsoft Teams, (well 9 of 11, DB and SAM photos in projects), Dr. Joanna Rutkowska bottom right

Research topics proposed and chosen by participants

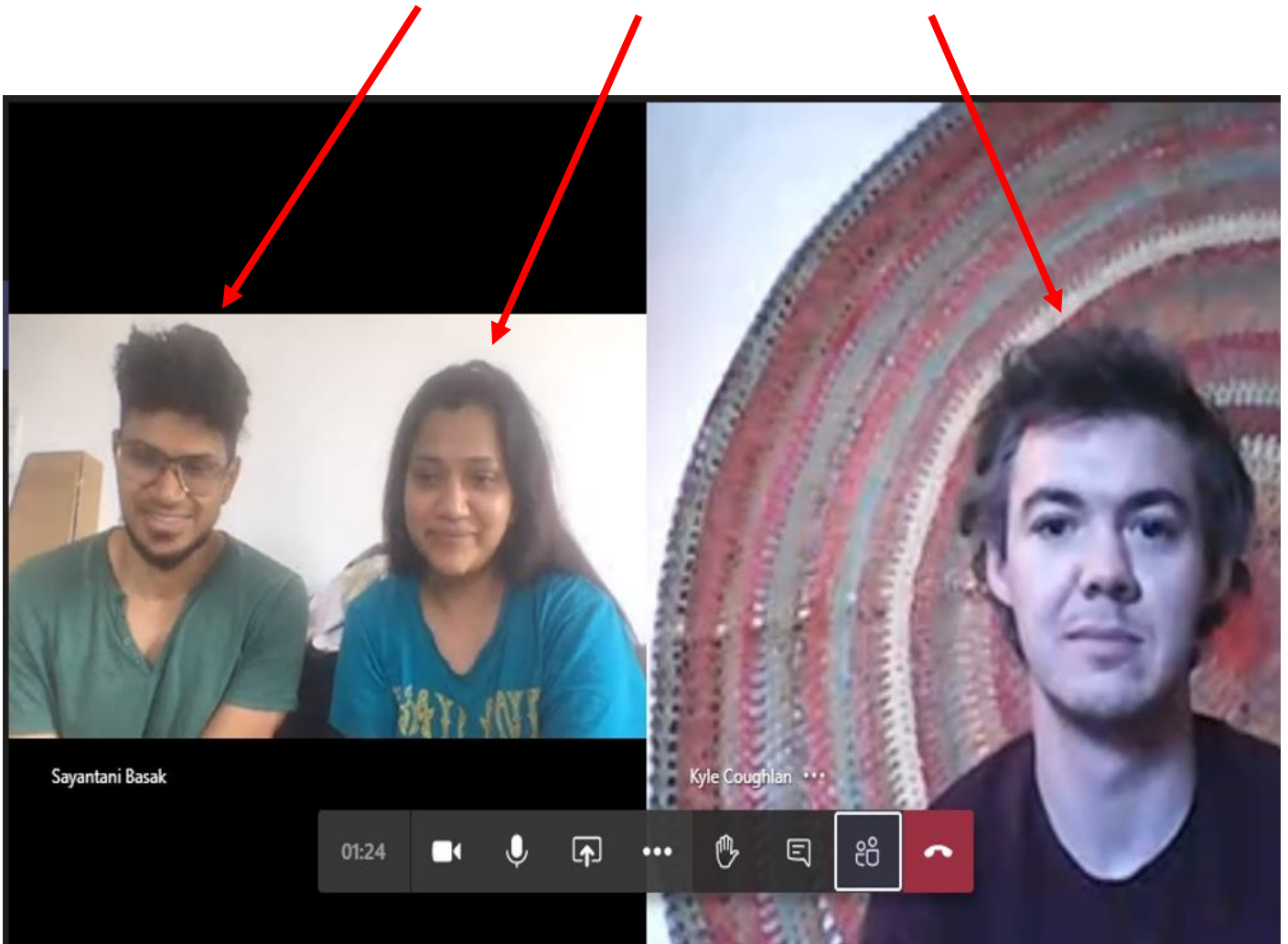
- Are artificial sweeteners more harmful than refined sugars? (SB)
- Does ketogenic diet improve health quotient? (AKB)
- **Is the morning breakfast the most important meal of the day? (AKB)**
- Depression and anxiety during global pandemic (AKB)
- **Whether human abilities of face recognition changes during Covid-19 pandemic? (TG)**
- Whether pro-ecological habits affect long-term happiness? (TG)
- **Is a sense of symmetry an innate state? (TG)**
- How far/long can insects fly? (SAM)
- Does space travel impact fertility and fecundity of insects? (SAM)
- Tree health in the cities (SAM)
- Does whole-body cryotherapy retard aging? (SH)
- Effects of multiple Covid-19 infections on spermatogenesis (SH)
- Is there an association between iris heterochromia and hair colour? (SH)
- How does selection history affect costs of gaining adaptations? (MO)
- Whether quorum sensing molecules influence the structure of the colony? (MO)
- How does hormonal therapy affect microbiome? (MO)
- Identifying human-animal interactions in older adults (SB)
- Does choice of beverage impact work hours? (SB)
- **Influence of flora on balconies on insect abundance (MO-C)**
- Ants and wasps as potential vectors of Nosema parasite (MO-C)
- The impact of artificial red mason bees' nests on abundance of other species (MO-C)
- Effects of isolation in parental care and chick development in a gregarious songbird, zebra finches? (KC)
- Sexy son hypothesis: female promiscuity and male parental care giving in great tits (KC)
- Covid-19 and climate change: reduction on CO₂ output but lowering public awareness? (KC)
- Impact of logging devices on bird's behaviour and breeding success (DB)
- Psychiatric disorders in Covid-19 survivors (DB)
- Conservation of endangered rhinos by checking animal droppings (DB)
- Factors affecting physical and psychological conditions of animals in zoological gardens (ESz)
- The effect of light pollution on sleep behaviour in free-living birds (ESz)
- The role of earlier eclosion on their Drosophila fitness (flight performance, fecundity) (ESz)
- The effect of mitochondria introgression on fitness in amphibians (PJ)
- The influence of urbanisation on the prevalence of pathogenic fungus Batrachochytrium dendrobatidis (PJ)
- The mysteries of contact zone of Anguis sp (PJ)

* Projects in bold were later chosen as research topics

Project 1:

Is the morning “breaking of fast” the most important meal of the day? Intermittent fasting and its role in weight management

Arpan Kumar Basak, Sayantani Basak & Kyle Coughlan



Draft project proposal

Title: Is the morning “breaking of fast” the most important meal of the day? Intermittent fasting and its role in weight management.

Applicants: Kyle Coughlan, Arpan Kumar Basak and Sayantani Basak

Summary

BACKGROUND

The scope of the obesity epidemic cannot be underestimated. It has a direct impact on the long term mobility as well as the development of many diseases including type-2 diabetes, high blood pressure, high cholesterol, atherosclerosis, musculoskeletal disorders, and even cancers. Consequently, a huge amount of money is spent annually on the health and fitness industry, sometimes without any significant result. A fast-growing diet plan namely Intermittent Fasting has gained popularity in recent years, which involves skipping morning breakfast. Through this project, we would like to find out the effect of Intermittent Fasting as a more economical, non-pharmacological approach to weight loss and improvement of overall public health.

RESEARCH QUESTION AND HYPOTHESES

To address the impact of Intermittent fasting we plan to ask are whether skipping a meal, in this case, breakfast, help with weight management and whether prolonged intermittent fasting can lead to overall health improvement. We will test three hypotheses stating that skipping morning breakfast will reduce modified body mass index, prolonged fasting improves the movement activities, and the blood and lipid profile of the treated individuals will improve to optimum levels throughout prolonged fasting.

METHODS

To test these three hypotheses, the study will be conducted on 300 volunteers belonging to the age group between 30-45 years, leading a sedentary life with no major physical activity (e.g. exercises) with Body Mass Index over 25 (overweight). The 300 individuals will be then divided into 3 groups, one control group and two fasting groups of two different fasting hours. The experiments will continue over 12 weeks, conducted once in winter 2021 and repeated in summer 2022. The biochemical analysis will be conducted on the blood samples collected before initiation of the experiment and after completion of the experiment.

RELEVANCE

The finding of this study can contribute to our understanding of metabolic health and weight loss and can assist in developing programs globally to reduce obesity levels and saving money used on treatment or commercial dietary supplements.

Short description

1. Scientific goal of the project

Obesity is an epidemic which is worsening in most parts of the world and one which has major implication in regard to both global health and the global economy (Gregg and Shaw, 2017) with the World Health organisation recording a 3 fold increase in obesity between 1975 and 2016. Overweight and obesity are linked significantly to the development of many diseases including type-2 diabetes, high blood pressure, high cholesterol, atherosclerosis, musculoskeletal disorders, and some cancers. These in turn lead to coronary heart disease (CHD), cardiovascular disorders (CVD), and stroke (Chu et al. 2018), and obesity has also recently been shown to be a significant risk factor for the development of server COVID-19 infections (Scatter et al.

2020). The development of this obesity crisis has been linked to changing dietary habits, with the rate of consumption of sugars increasing greatly (Johnson et al. 2017). Another societal change is linked to obesity is the prevalence of sedentary working conditions, namely the 9 to 5 office job. This type of employment in which people sit for long periods in a sedentary posture has been significantly associated with higher rates of CVD and increased body mass index (BMI) (Tigbe et al. 2017). We wish to test an eating regime which is growing in popularity, known as **intermittent fasting (IF)** which works behind the idea of skipping breakfast in the morning. However, it does not imply any loss of calorie intake throughout the day. IF simply follows the ideology that one should have a strict eating period than munching throughout the day. This food intake restriction schedules cycle between voluntary fasting and non-fasting over a given period. Clinical trials in humans and animals have shown that IF elicits evolutionary conserved, adaptive cellular responses that improve glucose regulation, increase stress resistance, and suppress inflammation (de Cabo and Matteson 2019). It has been shown that during periods of fasting, fatty acids are produced by breaking down triglycerides stored as fat in adipose tissue. These are converted into ketone bodies by the liver which are used as a major source of energy for many tissues including the brain. Normally, in a fed state, ketone body levels are low in the blood but begin to rise within 8 to 12 hours of fasting and continue to rise even up to 48 hours post feeding (Browning et al. 2012). These clinical results seem to suggest that IF is beneficial, however most people eat 3 meals a day, plus snacks and so IF does not occur and this “metabolic switch” remains off. The research questions we wish to ask are (RQ1) whether skipping a meal, in this case breakfast, can help with weight management and (RQ2) whether prolonged intermittent fasting can lead to an overall health improvement. To answer these two questions, we will test 3 hypotheses **(H1) Skipping morning breakfast will reduce modified body mass index (mBMI, conventional BMI with liquid retention factored in)**, **(H2) Prolonged fasting improves the movement activities** and **(H3) The blood and lipid profile of the treated individuals will improve to optimum levels over the course of prolonged fasting**.

Intermittent fasting has proven to promote weight loss and improve metabolic health (Patterson and Sears, 2016) and while real world empirical data is still scarce if proven efficacious these sorts of eating regimes may provide an economical, non-pharmacological approach to weight loss and improving of overall public health which is why we wish to undertake this experiment.

2. Significance of the project

2.1 State of the art

The scope of the obesity epidemic cannot be underestimated. According to the WHO, in 2016, 1.9 billion adults were overweight, with 650 million being obese, with rates showing an upward trend. Developed as a solution, IF has grown over the past 10 to 15 years as an unconventional approach to potentially reduce body weight and improve metabolic health beyond simple calorie restriction (CR) (Hoddy et al., 2020). IF has been used as the umbrella term to define the eating patterns in which individuals undergo extended fasting time periods (e.g., 16 h), with intervening periods of normal food intake, on a recurring basis (Anson et al., 2003). Nearly all IF studies have resulted in some degree of weight loss, ranging from 2.5–9.9% and associated fat mass loss (Byrne et al., 2018; Heilbronn et al., 2005). A study reported (Klempel et al., 2012) found slightly greater weight reduction after 8-week intervention in IF amongst women with overweight and obesity. IF trials demonstrate great heterogeneity in design, with fasting periods initiated at different times of the day (e.g. 20-hour fasts from 10 pm to 6 pm (Hoddy et al., 2020), 24-hour fasts from midnight (Heilbronn et al., 2005) or 8 am (Hutchison et al., 2019) or ~18 hour fasts broken by small meals consumed at lunch or dinner. However, the optimal time to initiate the fasting period has not been studied. Reported metabolic benefits of IF with calorie restrictions include reduced cancer, improved insulin signalling, reduced incidence of stroke, neuroprotection, and increased lifespan (Anson et al., 2003). However, we plan to keep the calorie count constant to understand the impact of IF over the 12-week experimental term.

2.2 Project justification and impact

The World Obesity federation estimates that by 2025 annual global health spending due to obesity will be 1.2 trillion dollars. It is a problem which poses a substantial economic burden to governments of both developed and developing countries (Tremmel et al. 2017) and to individuals, with it estimating that

overweight and obese people spend 32% more on medical costs (Yusefzadch et al. 2019), not to mention the severe impact on health and overall well-being. It is estimated that in 2019 people spent 96.7 billion dollars globally on health and fitness club memberships and a recent market analysis report predicts that by the year 2027 the global market for dietary supplements will reach 230 billion dollars annually.

If this project is successful in discerning the health benefits of IF on a small sample of people, it opens up the possibility of implementing larger, longer term studies. The finding of this study can contribute to our understanding of metabolic health and weight loss and can assist in developing programs globally with the aim to reduce obesity levels and save money used on treatment or commercial dietary supplements. While there is much anecdotal evidence and personal praise for IF, empirical evidence is scarce which is why we wish to develop and design a robust experimental set up to test for the effects of IF on weight loss, metabolic health and energetics.

3. Concept and Work Plan

3.1 General work plan

The project is scheduled for 3 years (2021- 2023) including preparation of manuscript and attending conferences.

Choosing individuals and program setup (Research task RT1): Using social media, we will advertise the program to obtain volunteers for the experiments using online survey webtool. The information will have confidential questions about biodata, working hours, and health status. The selection criteria will be individuals belonging to the age group 30-45 years, working for 0900-1700 h, with no major physical activity (e.g. exercises) with BMI over 25. Among the respondents that meet the conditions specific to the criteria, we will select randomly 300 volunteers (150 male and 150 female). The 300 individuals will be divided into 3 groups. The first group with 100 volunteers will serve as the control group with no fasting periods. The second group (16_8) with 100 volunteers will serve 16 h of fasting period and 8 h of feeding period. The last group (20_8) with the remaining 100 volunteers will follow 20 h of fasting period and 4 h of feeding period. All selected volunteers will attend a meeting about the project and work plan. After the meeting volunteers will authorise the legal consent and ethical documentation. After authorisation, we will hand over the wearable device and collect the blood samples for data collection prior to the start of the experiment for biochemical analysis.

Daily and periodic observations (Research task RT2): Meals having constant calories and macronutrient profile will be distributed in 3 plates (outsourced: Catering services). The volunteers from the control group will receive the meals within 0800-0900 h, 16 h fasting group within 1200-1300 h and 20 h fasting group within 1500-1600h. The liquid intake and attitude towards the program will be noted daily using an online questionnaire. On completion of every 4-week period, the individual's body mass parameter will be estimated and their attitude towards the program will be assessed from the online questionnaire. The assessment is crucial to understand the change in psychological status of the individual in real-time and estimate the adaptation phase.

Biochemical analysis of blood (Research task RT3): After completion of the 12-week period, body mass parameters and blood samples will be collected from individuals belonging to the respective group. The biochemical analysis will be conducted on the blood samples taken from the individuals after the 12 weeks of prolonged fasting (outsourcing: SCANMED facility).

3.2 Specific research goals

1. Track the modified mBMI as a measure of weight management during program (RT2)
2. Record the activity along the course of program (RT2)
3. Profiling blood sample for hepatic, hormonal response, and lipid response for symptomatic variations before and after implementation of prolonged fasting. (RT1 and RT3)

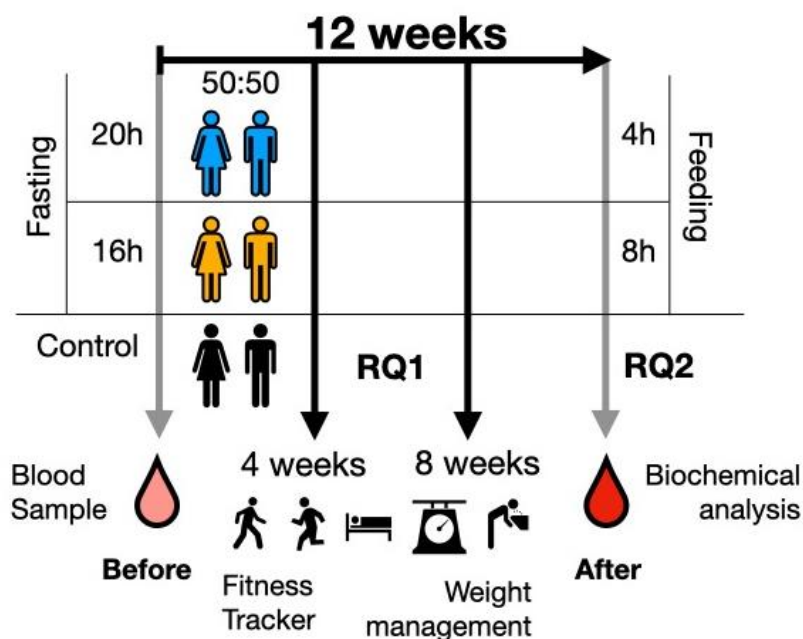


Figure 1. Proposed workflow for 12-week experiment

3.3. Preliminary result

Recent studies (Cho et al., 2019; Corley et al., 2018) have reported significant weight reduction (6%-8%) over an 8 to 12 week trial period following IF. However, these studies considered alternating the calorie intake or involving exercises. However, we would not consider calorie reduction and any major changes in the volunteer's lifestyle.

3.4 Risk analysis

The risk factors will be primarily determined from the assessment of online questionnaire as mentioned in RT2. There is a possibility that few individuals may deviate from the fasting group. In that case we will include the individuals to have robustness in our data. Volunteers that fail to maintain the daily calorie limit will also be considered during the time of analysis. We will consider the possibility of the individuals taking prolonged adaptation period with the program over 4 weeks and include as a factor in our dataset. However, in the situation where volunteers compromise the honour by misusing the fitness band, we will exclude the participant immediately from the experiment that tests H2.

4. Research Methodology

Underlying scientific methodology: The randomly selected 300 individuals meeting the criteria will then be provided with detailed program including meal plan. The total calorie intake in a 24 h period will be kept within to 2200-2400 Cal and nutrient intake will be in the range of 60-65% of Carbohydrates, 25-30% Proteins and 10-15% Fats. The content of the meal will be designed by consulting a nutritionist and coordinating with the catering services. The dietary habits will be considered and accordingly meals will be prepared. The fixed factors will be **group** (Control and fasting: 16_8 fasting: 20_h) and **sex** (M and F) with all other factors (e.g. age, working hours, health status, diet preference, adaptation period etc.) as random effects. Experiments will be conducted twice in a 2 year period to have a robust estimation of the population within two seasons (winter of 2021 and summer of 2022) to detect any seasonal variation. The second experiment will be conducted with a new set of individuals. In total, the dataset will have 600 sample representatives of overweight population.

During the course of the program: Measurements of body mass parameters will be conducted by the project members every 4th week. Daily supplementation of the meals will be provided prior to the breakfast as described in RT2. Online questionnaire will be sent to the individuals to share the attitude towards the program. The design of the questionnaire will be in a way that we can assess the possibility of the risk factors (e.g. failure to document step counts). Also, the daily liquid intake will be noted in the questionnaire. After a

period of 4 weeks, the body mass measurements will be taken. The log of daily activities including steps, calorie burnt, heart rate, steps, and sleep cycle will be tracked by Mi band (or similar device).

Biochemical analysis of the blood profile: Blood samples will be collected from each individual before and after the program is initiated. These blood samples will be used to estimate the hormonal level: thyroid stimulating hormone (TSH), serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), Uric Acid, Macro molecules, serum albumin and lipid content: including Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Triglycerides (TG). These parameters are markers of health conditions leading to obesity as compared to the optimum levels (Lee and Siddiqui, 2020). Deviation from the optimum level will state the health condition of the individual. Improvement in health condition will be assessed if the values fall into the range of optimum level after successful accomplishment of the program.

Data analysis: All analysis will be conducted in R environment, using specific packages. To test the hypothesis H1, we will use the body mass measurements and liquid intake to compute the modified BMI. Pairwise comparison between the control group and fasting group with sex as a co-factor over the course of the program (CM, CF, 16_8M, 16_8F, 20_4M, and 20_4F) using linear regression for the response of BMI and similarly test H2 by considering the activity parameters individually. To test both hypothesis H2 and H3, we will conduct unconstrained multivariate analysis by Non-metric Multidimensional Scaling and Linear Discriminant Analysis on the dataset obtained from the fitness band, blood and lipid profile. We will use constrained ordination by using Canonical Correlation Analysis using *vegan* package in R. We will normalise the data from the blood and lipid profile of the individuals before implementing the program and use Pairwise PERMANOVA like test to compute the overall variance of the fasting groups relative to control group using *RVAdemoire* package in R.

Equipment and devices to be used in research: Tracking daily activities - We will provide the selected volunteers a fitness band (Mi band) only during the period of the experiment. Data from these bands will be recorded in the repository for individual volunteers. We will reuse the band by formatting the data for the next batch of experiment.

5. Literature

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Budget Table

No.	Item	Funds for each budget year (PLN)			
		2021	2022	2023	Total
1	Direct costs, including:				
	- Salaries and scholarships	54000	54000	54000	162 000
	- Equipment	36000	0	0	36000
	- Other direct costs	861 000	871 000	50000	1 782 000
2	Indirect cost	190 200	185 000	20800	396 000
	Total costs (1+2)	1 141 200	1 110 000	124 800	2 376 000

Breakdown of project costs including justification and relevance for the tasks in the project

1. Direct Costs

- Salaries and scholarships

Number of Principal Investigators: **3**

No.	Full name and Type of position	Employing entity	Project-related remuneration period (months)	Rate of salary pool/banding	Total salary cost on grant (PLN)	Type of employment
1	Mgr. inz. Arpan Kumar Basak	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
2	Mgr. Kyle Coughlan	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
3	Mgr. Sayantani Basak	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
Total		162 000				

- Equipment and devices cost – 36 000 PLN

Fitness tracking device: for activity tracking for 300 individuals x price of Fitbit (120 PLN) (300x120 PLN) – 36 000,00 PLN

- Other direct costs – 1 782 000 PLN

Materials: (expendable goods for direct use in the project) Cost – **1 512 000 PLN**

Meal: meals for 300 individuals for 2 experiments for 12 weeks x price of food (30PLN) (300x2x12x7x30 PLN) – 1 512 000 PLN

Outsourcing: (services ordered from entities external to the Host Institution) – **210 000 PLN**

1. Pathological laboratory for blood and lipid profiling: Test conduction before and after the experimental period (twice) for 2 experiments involving 300 volunteers x price of lab tests (150 PLN) (2x2x300x150 PLN) – 180 000 PLN
2. Consultation with a nutritionist: Twice consultation for 2 experiments (15000x2) – 30000 PLN.

Conferences and business trips (by members of the research team) – **30 000 PLN**

Participation in national and international scientific conferences (including diet, accommodation, travel and printing of posters). We are planning to attend XX European Public Health Conference in 2022 as well as American Occupational Health Conference (AOHC) in 2023 – 30000 PLN.

Other costs – **30 000 PLN**

Publications costs (THE LANCET Public Health – 20000 PLN, and BMC Public Health – 10000 PLN) – 30000 PLN.

Reviews

Prof. dr hab. Jan Kozłowski

The title: In my opinion it is adequate and interesting. Full stop should not appear at the end of the title.

Summary: Some journals require this form of summary. It helps to clearly present a contents of a paper or project. In general, this goal has been achieved, with one exception, of the important sentence “The 300 individuals will be then divided into 3 groups, one control group and two fasting groups of two different fasting hours.” Here the authors should be more specific, because “two different fasting hours” may mean, say, one group between 9 and 11, the second between 11 and 13. Without reading the method description in the main body of the proposal, I would not have an idea about the design, and even after reading methods I am not sure about the schedule of feeding.

The project: It is not my job to concentrate on details; I was asked for general overview of all four projects. I like the project. It can help to decide about feeding schedule that can improve health status of obese people. But I must add a warning: physiological differences between people are enormous, especially in their metabolic rates. If all participants will get the same amount of calories, it may be more than enough for some of them and not enough for others. Thus it may be difficult to disentangle the effect of calorie amount served and the effect of feeding schedule (i.e., the role of skipping breakfast). I would recommend measuring metabolic rate (BMR and during a moderate exercise) before the experiment to adjust the amount of food to individual demand. In the sentence “The volunteers from the control group will receive the meals within 0800-0900 h, 16 h fasting group within 1200-1300 h and 20 h fasting group within 1500-1600h.” it is not clear for me how meals are distributed during a day. It seems that all groups will get only one meal, but at different hours. I guess that the control group will get food at 8 o'clock for three meals, 16h fasting group at 12 for two meals, and 20h fasting group at 15 for only one meal. But this is only my guess.

Prof. dr hab. Szymon Zubek

1. Assessment of the scientific level of research or tasks to be performed

This up-to-date research project proposal concerns an eating regime which is growing in popularity, known as intermittent fasting (IF), and its effect on weight loss and health of humans suffering from obesity. The concept and objectives are precisely presented in the proposal. Workplan is clear and has well-structured research tasks. The experiments are adequately developed, well-integrated and appropriate for the accomplishment of the objectives of the project what will be reflected in suitable results likely to be published in mainstream journals.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

Although the investigations involving IF have been performed recently, the present proposal has high level of novelty due to the fact that the PIs are about to fill the gap in knowledge on the optimal time to initiate the fasting period.

3. Assessment of the project feasibility

Project is ambitious but feasible in the timeframe proposed. The structure of the research team, including specialists-consultants, is correct. Some equipment necessary to conduct the study is planned to be purchased from the funds of the project. The Authors recognized potential risks well and provided a plan on what to do in the case of failure at some stages of the project implementation.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

The costs are well justified with regard to the subject and scope of the research. The detailed justifications of particular positions of the budget are provided.

5. Strengths of the proposal

Timely project. Precisely prepared proposal. A detailed and well-structured work plan is given. The research methodology has been presented in a clear and concise manner. The collaboration with nutritionists has been established to complete some research tasks. Potential practical outcomes of the project in human health programs.

The PIs are very ambitious in selecting journals in which they are about to publish their results.

6. Weaknesses of the proposal

The short version of project proposal is of very good quality and presents numerous strengths and no weaknesses.

Tomasz Gaczorek

1. Assessment of the scientific level of research or tasks to be performed

I highly appreciate the proposed idea of a study. The obesity which is placed here in a center of reasoning is indeed one of the most dangerous civilization deceases. It is also undoubtedly and inherently related to all others. This experiment provides a potential solution to limit the above-mentioned problem significantly. Additionally, clearly shown economical aspect is another advantage of the presented project. However, I am not convinced whether the increased level of money expenditure on activities such as fitness club attendance are the indicators of the rate of obesity within a society. To be honest, it can be another way around.

The scientific reasons for the choice of particular obesity and general health indicators were fully justified. Also, the whole underlying mechanism of potential fasting impact was explained in great detail.

Stated hypotheses were generally clear and followed the logical pathway of reasoning. However, concerning H1 hypothesis I am wondering what is the timescale. I assume it is the time of the experiment but I did not find any explanation why 12 weeks were chosen. Moreover, I have some doubts about the meaning of "improvement" in H2 hypothesis. Improved activities can be longer, more frequent, more intensive etc.. I would suggest to clarify it.

The study design properly represents the testing of the analyzed hypotheses. The only information that is lacking is whether provided meals from the outsourcing companies will serve as the only food intake over a whole day. If so, I am wondering whether you can distinguish the effect of fasting from the effect of calorie limitation. Additionally, I would suggest to record and examine the effect of volunteers' sleep length. According to my knowledge, it is thought to be one of the main factors inducing obesity propensities.

Proposed statistical methods are properly chosen and ensure that data analyses will be appropriately performed.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

As clearly stated in the main body of the proposal, obesity is one of the biggest challenges of a modern world. It generates tremendous costs including material ones as health services and ethical as individuals' suffering. The importance of the field makes this study extremely promising. The idea of fasting introduction as a way of reducing overweight provides the basic benefit of no additional methodological cost on the side of the patient. The study will also broaden our understanding of traditional and unconventional medicine which often promotes fasting. To conclude, I agree with the authors that it has great potential and that the research outcomes are going to be published in the leading journals.

3. Assessment of the project feasibility

The main risk associated with the presented project is related to the cooperation with the volunteers. I have not noticed any step of validation of information received from the participants. Generally, obese people tend

to be ashamed of overeating. This can potentially prevent some of them from honest reporting. Furthermore, I am aware that the authors wanted to make their results as universal as possible, that is why the research is not restricted to any geographic region. It can work perfectly but I would rather prefer to have some supervision. Especially if the expenses per individual participant (mainly daily food provision) are significant. Nevertheless, even reporting those two caveats, I assess the risk to be rather low. Honestly, I need to admit that I really like the idea of combining the experimental approach with the online surveys.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

From my point of view, the expenses are slightly underestimated. It is quite surprising to see such low PIs' salaries in comparison to the amount of work required and the level of scientists that submitted this project. Also, the price of an individual meal seems to be higher in practice, especially when it needs to contain a given number of calories. Probably also, no huge discounts from catering companies can be received as participants will be scattered around the world. Apart from that, I am wondering whether free meal and/or eventual perspective of weight loss is enough to encourage people to fast or whether it will not generate some bias toward the specific economic groups.

5. Strengths of the proposal

- The great importance of the topic which would be of the interest of a broad audience
- Potential research outcomes that can be directly adopted by the dietitians.
- A detailed description of research significance
- Clear and well-justified experimental design
- Low risk of the experiment failure or extension

6. Weaknesses of the proposal

- The project is heavily dependent on volunteers' behavior
- The improvement in mobility and the number of daily meals are not explicitly stated
- The costs seem to be underestimated

Sylwia Herman

1. Scientific level of research or tasks to be performed

All three hypotheses are well-framed, verifiable, eye-catching, and relevant to research questions. The research objective is explained. Experiments seem to be well-planned. However, they have some faults. Your Work plan is very detailed and well-organized, it looks professional. Nevertheless, the information about the area of participants' enlistment is missing, place of research, and its extent to other countries as well. I have mixed feelings about the length of the fasting period. You need to remember that it can have some negative side effects e.g. tiredness, insomnia, dizziness, psychological problems. Consequently, you may try to slightly modify the proportion of the fasting period to the feeding period or emphasize you will include a warning about the possibility of the above-mentioned implication occurrence. It is highly professional you have taken into consideration the legal consent authorization and you have made a decision about up to date checking the psychological status of the participants. The data analysis description is written in great detail.

2. Project's innovative potential and impact on the advancement of the scientific field/discipline

I have found your project crucial, original, and interesting. Suggested research concerns one of the civilization diseases that can lead to a wide range of dangerous complications and premature death and you demonstrated the current state of the art in this field. You showed there is an empirical data gap about the influence of Intermittent fasting on metabolic health as well. You demonstrated that the above-mentioned feeding problem is prevailing and there is a high necessity of dealing with it. This study actually can provide a new method of obesity level reduction. In my opinion, finding the economical solution is highly desirable and due to that, it

will be probably more encouraging to apply than a pharmacological one. The justification of the chosen research topic is convincing and supported numerous literature.

3. Feasibility

I am of the opinion your project is possible to perform. You have assessed the risk and suggested potential problem solutions. Line 170- the possibility of prolonged adaptation with the program still allows you to test the hypotheses. Your risk analysis should also take account of a chance you will not gather enough volunteers based on the online survey, eventually, you will need 600 individuals. You may consider paying the symbolic remuneration to participants. Due to that you can encourage them to your online survey completing and accelerate the research phase. What is more, the experiments seem to be exhausting. In terms of that, it would be difficult to find a volunteer for free. Line 168-it is not clear for me- which individuals will you include then?

4. Justification of the costs Costs of the project seem to be well-justified with regard to the subject and scope of the research.

5. Strengths of the proposal

- logical structure
- visually clear form of text
- well-written hypotheses
- novel approach to the topic and employment of modern devices
- project duration is apt
- big, representative sample of volunteers
- detailed Data analysis description

6. Weaknesses of the proposal

- undefined area of research carrying out and volunteer enlistment
- lack of internationality character
- preliminary results- in that paragraph you have demonstrated the current state of the art in your study topic. Probably here should be mentioned your preliminary small research or, if you claim it is not possible for your study to do this, maybe you should just skip this paragraph or write “not applicable”.

I would like to add that the title of your project is clear, arousing curiosity and reflecting your research topic. I need to say that I probably would not be able to repeat it from memory. Probably it would be catchier if it was slightly shorter. But I understand it is not the easiest task.

In the case of the summary of your project, it is full of information, explicit and clear, especially due to the division to the individual paragraphs. I would like to suggest to add keywords to your abstract. It was one of the first elements I started to look for in your document to see broadly your project pathway, its limits, and the main discussed topics. What is more, the short method description is misleading (line 26). After reading this I wasn't sure you will carry out experiments lasting 12 weeks in winter and 12 weeks in summer or you will conduct two experiments that in total last 12 weeks and you will divide this into 6 weeks in winter and 6 weeks in summer. Could you write it in other words because for me is not understandable enough in this part?

In addition, I really appreciate your figure 1. which pictures the proposed workflow for your experiment. I am of the opinion it is well-organized but because of so much information, it is a bit complicated. I needed a while to analyze it. I understand that the number of pages is limited but maybe try to make this figure broader to leave more space between particular time points.

To sum up, your grant proposal has a logical structure, is well-planned and easy to follow. The scientific language you have used is at a high level as well. However, there are some minor literal or grammar errors. Due to the well-organized text, in stages, there were appearing questions in my mind, and in most, with each following part, I could find an answer to them. Great job! 🍀

Final project proposal

Title: Is the morning “breaking of fast” the most important meal of the day? Intermittent fasting and its role in weight management

Applicants: Kyle Coughlan, Arpan Kumar Basak and Sayantani Basak

Summary

BACKGROUND

The scope of the obesity epidemic cannot be underestimated. It has a direct impact on mobility as well as the development of many diseases including type-2 diabetes, high blood pressure, high cholesterol, atherosclerosis, musculoskeletal disorders, and some cancers. Consequently, a huge amount of money is spent annually on the health and fitness industry, sometimes without any significant result. A fast-growing diet plan, namely Intermittent Fasting, has gained popularity in recent years which involves skipping morning breakfast. Through this project, we would like to find out the effects of Intermittent Fasting as a more economical, non-pharmacological approach to weight loss and improvement of overall public health.

RESEARCH QUESTION AND HYPOTHESES

To address the impact of Intermittent fasting we plan to ask are whether skipping a meal, in this case, breakfast, can help with weight management and whether prolonged intermittent fasting can lead to overall health improvement. We will test three hypotheses stating that skipping morning breakfast will reduce modified body mass index, prolonged fasting improves the movement activities, and the blood and lipid profile of the treated individuals will improve to optimum levels throughout prolonged fasting.

METHODS

To test these three hypotheses, the study will be conducted on 300 volunteers belonging to the age group between 30-45 years, leading a sedentary life with no major physical activity (e.g. exercises) with a Body Mass Index over 25 (overweight). The 300 individuals will be then divided into 3 groups, one control group and two fasting groups of two distinct fasting hours and specific feeding hours. The experiments will continue over 12 week period, conducted once in winter 2021 and repeated in summer 2022, for a total of 600 test subjects and based in the city of Krakow, Poland. Biochemical analysis will be conducted on the blood samples collected before initiation of the experiment and after completion of the experiment.

RELEVANCE

The finding of this study can contribute to our understanding of metabolic health and weight loss and can assist in developing programs globally to reduce obesity levels and thus potentially reducing resources used on medical treatments or commercial dietary supplements.

Short description

1. Scientific goal of the project

Obesity is an epidemic which is worsening in most parts of the world and one which has major implication in regard to both global health and the global economy (Gregg and Shaw, 2017) with the World Health organisation recording a 3 fold increase in obesity between 1975 and 2016. Overweight and obesity are linked significantly to the development of many diseases including type-2 diabetes, high blood pressure, high cholesterol, atherosclerosis, musculoskeletal disorders, and some cancers. These in turn lead to coronary heart

disease (CHD), cardiovascular disorders (CVD), and stroke (Chu et al. 2018), and obesity has also recently been shown to be a significant risk factor for the development of severe COVID-19 infections (Scatter et al. 2020). The development of this obesity crisis has been linked to changing dietary habits, with the rate of consumption of sugars increasing greatly in recent decades (Johnson et al. 2017). Another societal change linked to obesity is the prevalence of sedentary working conditions, namely the 9 to 5 office job. This type of employment in which people sit for long periods in a sedentary posture has been significantly associated with higher rates of CVD and increased body mass index (BMI) (Tigbe et al. 2017). We wish to test an eating regime which is growing in popularity, known as **intermittent fasting (IF)**, which is based on the idea of skipping breakfast in the morning. However, it does not imply any loss of calorie intake throughout the day. IF simply follows the ideology that one should have a strict eating period rather than eating throughout the day. This food intake restriction schedule cycles between voluntary fasting and non-fasting over a given period. Clinical trials in humans and animals have shown that IF elicits evolutionary conserved, adaptive cellular responses that improve glucose regulation, increase stress resistance, and suppress inflammation (de Cabo and Matteson 2019). It has been shown that during periods of fasting, fatty acids are produced by breaking down triglycerides stored as fat in adipose tissue. These are converted into ketone bodies by the liver which are used as a major source of energy for many tissues including the brain. Normally, in a fed state, ketone body levels are low in the blood but begin to rise within 8 to 12 hours of fasting and continue up to rise even up to 48 hours post feeding (Browning et al. 2012). These clinical results seem to suggest that IF is beneficial, however most people eat 3 meals a day, plus snacks and so IF does not occur and this “metabolic switch” remains off. The research questions we wish to ask are (RQ1) whether skipping a meal, in this case breakfast, can help with weight management and (RQ2) whether prolonged intermittent fasting can lead to an overall health improvement. To answer these two questions, we will test 3 hypotheses (**H1**) **Skipping morning breakfast will reduce modified body mass index (mBMI, conventional BMI with liquid retention factored in).** (**H2**) **Prolonged fasting improves the movement activities** and (**H3**) **The blood and lipid profile of the treated individuals will improve to optimum levels over the course of prolonged fasting.**

Intermittent fasting has shown to promote weight loss and improve metabolic health (Patterson and Sears, 2016) and while real world empirical data is still scarce, if proven efficacious these sorts of eating regimes may provide an economical, non-pharmacological approach to weight loss and improving of overall public health which is why we wish to undertake this experiment.

2. Significance of the project

2.1 State of the art

The scope of the obesity epidemic cannot be underestimated. According to the WHO, in 2016, 1.9 billion adults were overweight, with 650 million being obese, with rates showing an upward trend. Developed as a solution, IF has grown over the past 10 to 15 years as an unconventional approach to potentially reduce body weight and improve metabolic health beyond simple calorie restriction (CR) (Hoddy et al., 2020). IF has been used as the umbrella term to define the eating patterns in which individuals undergo extended fasting time periods (e.g., 16 h), with intervening periods of normal food intake, on a recurring basis (Anson et al., 2003). Nearly all IF studies have resulted in some degree of weight loss, ranging from 2.5–9.9% and associated fat mass loss (Byrne et al., 2018; Heilbronn et al., 2005). A study reported (Klempel et al., 2012) found slightly greater weight reduction after 8-week intervention in IF amongst women with overweight and obesity. IF trials demonstrate great heterogeneity in design, with fasting periods initiated at different times of the day (e.g. 20-hour fasts from 10 pm to 6 pm (Hoddy et al., 2020), 24-hour fasts from midnight (Heilbronn et al., 2005) or 8 am (Hutchison et al., 2019) or ~18 hour fasts broken by small meals consumed at lunch or dinner. However, the optimal time to initiate the fasting period has not been studied. Reported metabolic benefits of IF with calorie restrictions include reduced cancer, improved insulin signalling, reduced incidence of stroke, neuroprotection, and increased lifespan (Anson et al., 2003). However, we plan to keep the calorie count constant to understand the impact of IF over the 12-week experimental term.

2.2 Project justification and impact

The World Obesity federation estimates that by 2025 annual global health spending due to obesity will be 1.2 trillion dollars. It is a problem which poses a substantial economic burden to governments of both developed and developing countries (Tremmel et al. 2017) and to individuals, with it estimated that overweight and obese people spend 32% more on medical costs (Yusefzadch et al. 2019), not to mention the severe impact on health and overall well-being. It is estimated that in 2019 people spent 96.7 billion dollars globally on health and fitness club memberships and a recent market analysis report predicts that by the year 2027 the global market for dietary supplements will reach 230 billion dollars annually.

If this project is successful in discerning the health benefits of IF on a small sample of people, it opens up the possibility of implementing larger, longer term studies. The findings of this study can contribute to our understanding of metabolic health and weight loss and can assist in developing programs globally with the aim to reduce obesity levels and save money used on treatments or commercial dietary supplements. While there is much anecdotal evidence and personal praise for IF, empirical evidence is scarce which is why we wish to develop and design a robust experimental set up to test for the effects of IF on weight loss, metabolic health and energetics.

3. Concept and Work Plan

3.1 General work plan

The project is scheduled for 3 years (2021- 2023) including preparation of manuscript and attending conferences.

Choosing individuals and program setup (Research task RT1): Using social media, we will advertise the program to obtain volunteers for the experiments using an online survey webtool in Krakow, Poland. The information will have confidential questions about biodata, working hours, and health status. The selection criteria will be individuals belonging to the age group 30-45 years, working for 0900-1700 h, with no major physical activity (e.g. exercises) with BMI over 25. Among the respondents that meet the conditions specific to the criteria, we will select randomly 300 volunteers (150 male and 150 female). The 300 individuals will be divided into 3 groups. The first group with 100 volunteers will serve as the control group with no fasting periods. The second group (16_8) with 100 volunteers will serve 16 h of fasting period and 8 h of feeding period. The last group (20_8) with the remaining 100 volunteers will follow 20 h of fasting period and 4 h of feeding period. All selected volunteers will attend a meeting about the project and work plan. After the meeting volunteers will authorise the legal consent and ethical documentation. After authorisation, we will hand over the wearable device, measure the Basal Metabolic Rate (BMR) and collect the blood samples for data collection prior to the start of the experiment for biochemical analysis.

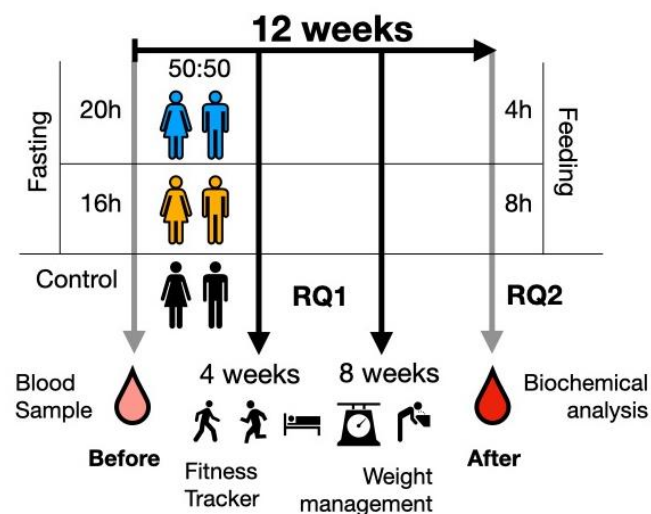


Figure 1. Proposed workflow for 12-week experiment

Daily and periodic observations (Research task RT2): Meals having constant calories and macronutrient profile will be distributed (outsourced: Catering services). Volunteers from the three groups will each receive 3 meals per day. The control group will receive the meals within 0800-0900 h, 16 h fasting group within 1200-1300 h and 20 h fasting group within 1500-1600h. The liquid intake and attitude towards the program will be noted daily using an online questionnaire. On completion of every 4-week period, the individual's body mass parameter will be estimated and their attitude towards the program will be assessed from the online questionnaire. The assessment is crucial to understand the change in psychological status of the individual in real-time and estimate the adaptation phase.

Biochemical analysis of blood (Research task RT3): After completion of the 12-week period, body mass parameters, BMR and blood samples will be collected from individuals belonging to the respective groups. The biochemical analysis will be conducted on the blood samples taken from the individuals after the 12 weeks of prolonged fasting (outsourcing: SCANMED facility).

3.2 Specific research goals

1. Track the modified mBMI as a measure of weight management during program (RT2)
2. Record the activity along the course of program (RT2)
3. Profiling blood sample for hepatic, hormonal response, and lipid response for symptomatic variations before and after implementation of prolonged fasting. (RT1 and RT3)

3.3. Preliminary result

Recent studies (Cho et al., 2019; Corley et al., 2018) have reported significant weight reduction (6%-8%) over an 8 to 12 week trial period following IF. However, these studies considered alternating the calorie intake or involving exercises. However, we would not consider calorie reduction and any major changes in the volunteer's lifestyle.

3.4 Risk analysis

The risk factors will be primarily determined from the assessment of online questionnaire as mentioned in RT2. There is a possibility that few individuals may deviate from the fasting group. In that case we will include the individuals to have robustness in our data. Volunteers that fail to maintain the daily calorie limit will also be considered during the time of analysis. We will consider the possibility of the individuals taking prolonged adaptation period with the program over 4 weeks and include as a factor in our dataset. However, in the situation where volunteers compromise the honour by misusing the fitness band, we will exclude the participant immediately from the experiment that tests H2.

4. Research Methodology

Underlying scientific methodology: The randomly selected 300 individuals meeting the criteria will then be provided with detailed program including meal plan. The total calorie intake in a 24 h period will be kept within to 2200-2400 Cal and nutrient intake will be in the range of 60-65% of Carbohydrates, 25-30% Proteins and 10-15% Fats. The content of the meal will be designed by consulting a nutritionist based on the BMR charts for individuals. The calorie intake will be distributed in 3 distinct meals in coordination with the catering services. The dietary habits will be considered and accordingly these meals will be prepared. The fixed factors will be **group** (Control and fasting: 16_8 fasting: 20_h) and **sex** (M and F) with all other factors (e.g. age, working hours, health status, diet preference, adaptation period etc.) as random effects. Experiments will be conducted twice in a 2 year period to have a robust estimation of the population within two seasons (winter of 2021 and summer of 2022) to detect any seasonal variation. The second experiment will be conducted with a new set of individuals. In total, the dataset will have 600 sample representatives of overweight population.

During the course of the program: Measurements of body mass parameters will be conducted by the project members every 4th week. Daily supplementation of the meals will be provided prior to the breakfast as described in RT2. Online questionnaire will be sent to the individuals to share the attitude towards the program. The design of the questionnaire will be in a way that we can assess the possibility of the risk factors (e.g. failure to document step counts). Also, the daily liquid intake will be noted in the questionnaire. After a period of 4 weeks, the body mass measurements will be taken. The log of daily activities including steps, calorie burnt, heart rate, steps, and sleep cycle will be tracked by Mi band (or similar device). The progress of mobility will be assessed based on improvement in heart-rate over the experimental period.

Biochemical analysis of the blood profile: Blood samples will be collected from each individual before and after the program is initiated. These blood samples will be used to estimate the hormonal level: thyroid stimulating hormone (TSH), serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), Uric Acid, Macro molecules, serum albumin and lipid content: including Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Triglycerides (TG). These parameters are markers of health conditions leading to obesity as compared to the optimum levels (Lee and Siddiqui, 2020). Deviation from the optimum level will state the health condition of the individual. Improvement in health condition will be assessed if the values fall into the range of optimum level after successful accomplishment of the program. Here, we will also estimate the BMR of the individuals between fasting groups before and after the program.

Data analysis: All analysis will be conducted in R environment, using specific packages. To test the hypothesis H1, we will use the body mass measurements and liquid intake to compute the modified BMI. Pairwise comparison between the control group and fasting group with sex as a co-factor over the course of the program (CM, CF, 16_8M, 16_8F, 20_4M, and 20_4F) using linear regression for the response of BMI and similarly test H2 by considering the activity parameters individually. To test both hypothesis H2 and H3, we will conduct unconstrained multivariate analysis by Non-metric Multidimensional Scaling and Linear Discriminant Analysis on the dataset obtained from the fitness band, blood and lipid profile. We will use constrained ordination by using Canonical Correlation Analysis using *vegan* package in R. We will normalise the data from the blood and lipid profile of the individuals before implementing the program and use Pairwise PERMANOVA like test to compute the overall variance of the fasting groups relative to control group using *RVAdemoire* package in R.

Equipment and devices to be used in research: Tracking daily activities - We will provide the selected volunteers a fitness band (Mi band) only during the period of the experiment. Data from these bands will be recorded in the repository for individual volunteers. We will reuse the band by formatting the data for the next batch of experiment.

5. Literature

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Budget Table

No.	Item	Funds for each budget year (PLN)			
		2021	2022	2023	Total
1	Direct costs, including:				
	- Salaries and scholarships	54000	54000	54000	162 000
	- Equipment	36000	0	0	36000
	- Other direct costs	861 000	871 000	50000	1 782 000
2	Indirect cost	190 200	185 000	20800	396 000
	Total costs (1+2)	1 141 200	1 110 000	124 800	2 376 000

Breakdown of project costs including justification and relevance for the tasks in the project

2. Direct Costs

- Salaries and scholarships

Number of Principal Investigators: 3

No.	Full name and Type of position	Employing entity	Project- related remuneration period (months)	Rate of salary pool/ banding	Total salary cost on grant (PLN)	Type of employment
1	Mgr. inz. Arpan Kumar Basak	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
2	Mgr. Kyle Coughlan	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
3	Mgr. Sayantani Basak	Faculty of Biology, Jagiellonian University, Krakow	36	1500/month	54000	Contract
	Project Tasks	Planning, preparing and conducting experiments, statistical analyses of obtained results; compilation of results and conclusions; presentations at scientific conferences; articles writing.				
Total		162 000				

- Equipment and devices cost – 36 000 PLN

Fitness tracking device: for activity tracking for 300 individuals x price of Fitbit (120 PLN) (300x120 PLN) – 36 000,00 PLN

- Other direct costs – 1 782 000 PLN

Materials: (expendable goods for direct use in the project) Cost – **1 512 000 PLN**

Meal: meals for 300 individuals for 2 experiments for 12 weeks x price of food (30PLN) (300x2x12x7x30 PLN) – 1 512 000 PLN

Outsourcing: (services ordered from entities external to the Host Institution) – **210 000 PLN**

1. Pathological laboratory for blood and lipid profiling: Test conduction before and after the experimental period (twice) for 2 experiments involving 300 volunteers x price of lab tests (150 PLN) (2x2x300x150 PLN) – 180 000 PLN
2. Consultation with a nutritionist: Twice consultation for 2 experiments (15000x2) – 30000 PLN.

Conferences and business trips (by members of the research team) – **30 000 PLN**

Participation in national and international scientific conferences (including diet, accommodation, travel and printing of posters). We are planning to attend XX European Public Health Conference in 2022 as well as American Occupational Health Conference (AOHC) in 2023 – 30000 PLN.

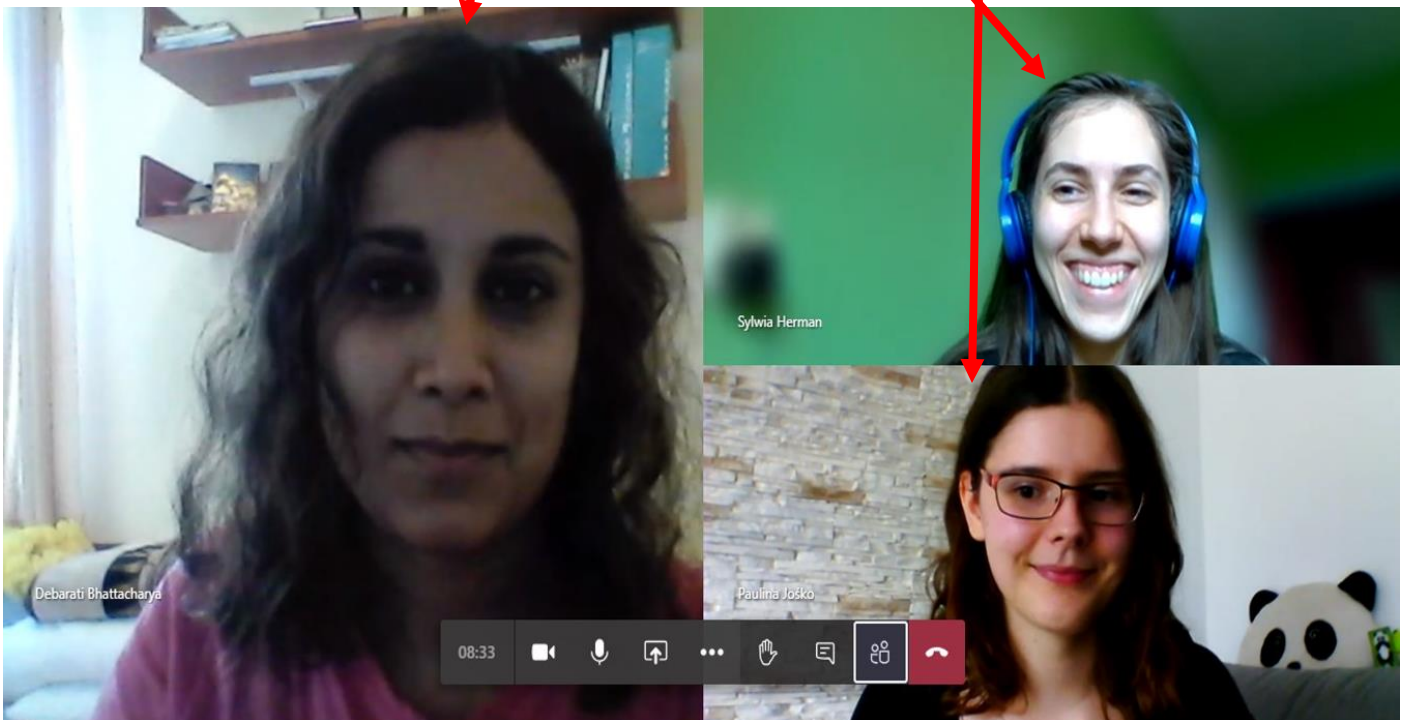
Other costs – **30 000 PLN**

Publications costs (THE LANCET Public Health – 20000 PLN, and BMC Public Health – 10000 PLN) – 30000 PLN.

Project 2:

Behind the mask. Human abilities to face recognition during the Covid-19 pandemic

Debarati Bhattacharya, Sylwia Herman & Paulina Joško



Draft project proposal

Title: Behind the mask. Human abilities of face recognition during Covid-19 pandemic.

Applicants: Debarati Bhattacharya, Sylwia Herman, Paulina Joško

Summary

The subject of face recognition is very important in social interactions. Facial recognition guides us to find clue about a person's emotions, personality, and gender. This recognition power is currently being challenged during the time of COVID-19 pandemic with majority of faces now being covered by masks. Unfortunately, still little is known about possible influence of other factors on recognition of faces that are covered. With intention to help to ease this global challenge, our study was designed to investigate the influence of age and sex on identifying covered faces and emotions during pandemic. The methods to be used for the study will be an online questionnaire followed by experimental tasks. In task one we will follow a Cambridge Face Memory Test (CFMT) modified by additional using masked images of a person. In task two the participants ability to identify emotions by looking at facial expressions on images will be tested. We also intend to check if previous experience of interacting with people wearing masks improved the ability of facial recognition, the issue that wasn't examined so far. We predict that women will perform better than men in recognizing faces and emotions and this ability will decrease with age in both task one and task two. While in societies with previous face covering history people could perform better with recognizing covered faces and emotions. We believe that the outcomes of this study can have positive impact on neurological and social sciences.

Keywords: facial recognition, emotions, face mask, pandemic, Covid-19

Scientific goal of the project

The ability to recognize people faces and emotions they are expressing is crucial skill in human communication and social interactions. Emotions are strongly connected with affectivity and pose a linkage between human bodies and their physical and social environment [Fox, 2015; Russell and Fernandez-Dols, 1997; Jingguang et al., 2010]. Nowadays we are facing a world-wide pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (Covid-19) that expresses itself with a wide variety of presenting symptoms in patients [Beaupre et al., 2020]. Due to droplet route of infection of the virus, a common way of preventing spreading the virus is using face mask to cover nose and mouth [Ashtiani et al., 2020; DOUNG-NGERN et al., 2020]. It is now impossible to predict when it will be safe again to restrict from the mask wearing regulations or the pandemic to be overcome. As a result, the societies all over the world are now facing a new challenge of interacting with others with their faces being covered by masks. There are some studies showing that the face covering may affect the ability to recognize other people and emotions they are expressing [e.g. Esteves and Öhman, 1993; Kret and Fischer, 2018]. However, they cover small study sample, therefore the analysis did not consider possible influence of other factors, such as sex or age of participants. Sudden emergence of totally new interrupting factor on a global scale creates unique opportunity for deeper and more detailed study of the issue with a completely novel approach. It is important to understand in greater detail which groups of people can be more susceptible to having difficulties due to this now worldwide issue. In our study, we will attempt to identify those more vulnerable groups. Additionally, we intend to investigate if it is possible for people to adapt to this new condition and improve the ability to recognize faces and emotions when they are more familiar with faces covered with masks, as it was not previously examined. The following hypotheses will be tested:

1. Females have higher ability to recognize covered faces than males.
2. The ability to recognize covered faces declines with age.
3. Females have higher ability to recognize emotions expressed on covered faces than males.
4. The ability to recognize emotions expressed on covered faces declines with age.
5. Societies with previous face covering history are better with recognizing covered faces and emotions they are expressing.

Significance of the project

There are some studies indicating that women outperform men in recognizing emotions based on facial expressions [Montagne et al., 2005; Wingenbach et al., 2018], as well as recognizing faces [Lewin and Herlitz, 2002; Weirich et al., 2011]. What is more, other studies on facial and emotion recognition reveal that those abilities decrease with age [Lamont et al., 2005; Sullivan et al., 2007; Mill et al., 2009; Abbruzzese et al., 2019].

However, in those studies, an additional factor of facial covering was not taken into consideration. Furthermore, those studies were conducted on the local scale, with a smaller study sample, thus the general applicability of those results can be questionable.

There are no studies examining if people are capable to improve those abilities after adjusting to face-covering with masks over time.

The suggested study can contribute to a better understanding of how sex, age, or previous experience of interacting with people wearing masks can impact on the human ability to recognize emotions and other people, by looking on their faces, covered and uncovered with the mask. It will help to indicate groups of people who can have limited above-mentioned capabilities and consequently can have difficulties with social interactions. What is more, our research will be useful in assessing if a longer exposition to interactions with masked people can facilitate face and emotion recognition.

To sum up our study outcomes:

- can help improving communication during the pandemic time.
- will have a positive impact of total inclusion of people from all spheres across the globe, projecting the theme- “WE ARE ONE AND WE ARE TOGETHER IN THE FIGHT”.
- can help further develop mental health care system, especially clinical implications leading to development of COVID mental health therapy and drug development.
- provide new insight to neurobiology and psychosociology.
- would be useful in clinical diagnosis and therapy development of several neuropsychiatric disorders.

Concept

We intend to conduct our study in 10 countries: the USA, Canada, United Kingdom, Spain, Italy, Germany, Poland, China, South Korea, Japan. We selected this list considering high Covid-19 incidences (according to WHO data) and the level of country development (common access to the internet is necessary). We included specific Asian countries, due to their previous history of people wearing face masks e.g. Japan [e.g. Burgess and Horii, 2012]. We plan to collaborate with other universities in those countries to help advertise information about our survey and collect data. We plan to compare obtained results based on the gender of volunteers, their age, or previous experience with social interactions with other people wearing face masks. We schedule the project duration for 1 year.

Research Goals

To address the issue of the influence of age and sex on the ability to identify faces and emotions during a pandemic.

To investigate whether people’s abilities to recognize faces and expressed emotions can improve when they are getting familiar with seeing mask-covered faces in their social interactions.

Work-plan

1. Designing a program that will display the questionnaire, preparatory task, and experimental task to participants in a form of an online survey. For this purpose, we will hire a programmer and provide him with all the details crucial for the study, that needs to be incorporated in the program.
2. Preparing our own database comprised of a wide range of digital images of human faces to display in tasks. To simplify this step, we could use already existing image databases but modify them, by covering the mouth and nose with a face mask. For this purpose, a professional graphic designer will be hired.
3. Finding potential collaborators in pandemic affected countries.
4. Advertising through local Govt pages, social media, health workers, NGOs, Religious institutions.

5. Collection of data – conducting the online survey.
6. Data analyzes.
7. Publishing results of our study in a form of scientific articles.

Risk analysis

Establishing new collaborations with universities from other countries can pose a challenge at the beginning of the project. Nevertheless, we believe that in each country we will be able to find collaborators, as the COVID-19 pandemic is a relevant and important issue on a global scale. Another difficulty we may encounter would be to not gather enough volunteers in each country. To prevent that, we will invest in advertising our study through various sources, as well as encourage people to participate in the survey, by offering cash remuneration. What is more, we will invest in translating the survey to languages other than English (depending on the country considered), to include non-English speaking volunteers.

Methodology

For our study, we will question group of volunteers from a range of countries affected by COVID-19. We will design an online survey consisting of a short questionnaire, preparative exercises, and a final experimental task. A short questionnaire will provide us with the necessary information about a volunteer (sex, age, country). We will also incorporate questions that enable us to exclude people with other disorders that can influence their performance of the test (such as visual imparity, autism, congenital prosopagnosia symptoms, psychiatric illness, alcohol or drug abuse). The preparative exercise is a necessary step to familiarize the volunteers with the computer program used in the study and task rules. This will be followed by the experimental task. Each volunteer will prepare for and perform task A and then task B. Both tasks rely on displaying various face images to the participant, an example of pictures that could be used for that purpose is presented in Fig. 1. The overall time for completing the survey should not exceed one hour. Randomly chosen participants (100 from each country) will be compensated for their effort with 22.00PLN per person. The survey will be prepared in English but, if necessary, can be translated.

For the age-based analyzes, we will assign obtained results to three age groups: A. 18-30, B. 31-50, C. 50+, according to the information provided by each participant in the questionnaire.



Fig.1. Exemplary pictures of faces with and without masks, that could be use in our study. Provided from <https://philadelphia.cbslocal.com/wp-content/uploads/sites/15116066/2020/08/face-mask-study.jpg>

Task description

Task A. To test hypotheses 1 and 2 we will design a task, in which participants are going to recognize faces from images presented to them. We can design our task based on the standard Cambridge Face Memory Test (CFMT; Duchaine and Nakayama, 2006), which is suitable for comparing age groups and genders (Cho et al., 2015). However, in our study, we will modify standard CFMT, by adding images of faces with masks covering the mouth and nose area.

Task B. To test hypotheses 3 and 4 we will design a task, in which participants are going to identify emotions expressed on faces presented to them on images. We will prepare a database of face images showing all basic expressions of emotion (happy, sad, angry, fearful, disgusted, surprised) and neutral expression, with an addition of a mask covering the area of mouth and nose. Each participant will be asked to identify emotion from the image presented, by choosing one of seven possible expressions listed below the image.

To test hypothesis 5, we will compare the results obtained in 3 selected Asian countries to the remaining 7 countries.

Results analysis

We will use Student's t-test while comparing 2 groups (e.g. men vs. women). In order to compare age groups, we will use a one-way ANOVA test. For all tests, Statistica 13 Software will be used, with significance level $p \leq 0.05$.

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Budget of the project

ITEM	Funds for each budget year (PLN)	
	2021	Total
1.Direct costs, including:		
1/Salaries and benefits	18000.00	18000.00
2/ Equipment	112318.8	112318.8
3/Other direct costs	56259.06	56259.06
2.Indirect costs	37315,57	37315,57
Total costs (1+2)	223893,43	223893,43

Breakdown of project costs including justification and relevance for the tasks in the project

1. Salaries and benefits:
 - Salaries for PI: 500 PLN per PI per month. Total cost: 18000 PLN. Main tasks of PI: planning, online survey preparing, the database of face images preparation, regular consultations with the programmer and professional graphic designer, statistical analyzes, publication writing, other essential work (documentation, organization, etc.).
2. Equipment:
 - Designing the software program: cost estimated as the time needed to develop the program, which is 3months, and cost per month which is 37,439.60 PLN. Final cost $37,439.60 \times 3 = 112318.8$ PLN (based on the current rate).
3. Other direct costs:
 - Hiring the professional graphic designer: cost estimated as the time needed to create a database of face images, which is 1 month, and cost per month, which is 12,105.60PLN.
 - Payment per person for participation in the survey: 22.00 PLN. We will pay 100 randomly selected participants from each of the 10 pandemic affected countries. In compliance with that, it amounts to $=100 \times 10 \times 22.00 = 22,000.00$ PLN for pay in total. The money transfer will be done through online services like PayPal to the respective participants.
 - Payment to university for translating the survey to languages other than English in non- English-speaking countries. We will fund 1 institute per country, a total of 10 institutes. Each institute will be paid 187.25 PLN for once to translate the questionnaire. Therefore, the total cost will be $187.25 \times 10 = 1872.5$ PLN. The cost includes the translation of the questionnaire by the language Centre.
 - Advertisement by health workers, NGOs, and religious institutions as 3,745.65 PLN per sector. Total cost: $3,745.65 \times 3 = 11,233.95$ PLN. For ads on Facebook, the cost for each ad is 26.93PLN per day. For a month of advertisement, it will be $26.93 \times 30 = 807.9$ PLN
 - Publication cost: in Health Journals the amount stands at 8239.11 PLN (based on the current rates).

Reviews

Prof. dr hab. Jan Kozłowski

The title is perfect in my opinion. But full stop should not appear at the end of the title.

Abstract: In general well written, although some information on planned methodology could be added. In the sentence “Unfortunately, still little is known about possible influence of other factors on recognition of faces that are covered.” it is not clear what “other factors” means, taking into account that factors were not mentioned in an earlier text.

The project: It is not my job to concentrate on details; I was asked for general overview of all four projects. In general, I like the project. I have, however, some objections, mainly technical. In English texts divisions to paragraphs are very important. Usually the first sentence is the main one, and rest of the paragraph is its explanation; or the last sentence is the main one, summarizing entire paragraph. Paragraphs should be longer than one sentence. Such rules are not satisfied in the part “Significance of the project.” Furthermore, I do not see the need for hiring a programmer for the level of complexity of the project (if necessary, one of investigators can learn programming in R; there is a lot of ready to use software that can be used); professional graphic designer to add a mask to existing pictures of faces seems also not necessary; I would recommend using this money to increase PI’s salary. I am not sure if wearing masks in Japan or Korea used to be so frequent in the past (before Covid-19 pandemic) to change people abilities that the authors are going to study. I would consider including a case of Muslim country where women cover faces since generations. This is not necessary to pay 10 institutions for translating a questionnaire: English is enough for three countries, and one of PI can prepare a Polish version. So only 7 institutions are necessary.

Prof. dr hab. Mariusz Cichoń

1. Assessment of the scientific level of research or tasks to be performed

This study is designed to check whether sex, age and previous experience affects the ability to recognize faces and basic emotions in humans in the situation when part of the face is hidden by a mask which is the case worldwide during the Covid-19 pandemics. To me the hypotheses presented in this proposal are derived *a'hoc*. I do not see good prerequisites that could justify such hypotheses. For instance, why one should expect individual age to affect face recognition? Is this because older people have more often problems with vision or due to ageing in neural system? Why do you expect differences between Japan and other countries as in most countries people wear mask already for at least half a year, so one could consider all countries as experienced? That could have sense if the study would be performed before pandemia. You mention that females are better in face recognition than males, so if you detect such sex difference in recognition of faces hidden behind the masks you would not know whether females are better to recognize hidden faces/emotions or just they are better recognizers regardless they are recognizing the unhidden or hidden faces.

I have difficulties in understanding the structure of the data that will be gathered, so it is difficult to judge the proposed data analyses. If you have binary data structure (recognized/not recognized) the proposed statistical tests might be the best option (if adequate at all).

2. Assessment of the project’s innovative potential and impact on the advancement of the scientific field/discipline

This sounds like a project in psychology. I am not an expert in this field, so I might not see its innovative potential. I do not see how anticipated effects of age and sex on the ability to recognize hidden

faces/emotions could have important impact of science and functioning of societies. Taking into account the existing knowledge.

3. Assessment of the project feasibility

The project is feasible. It is based on rather standard methods based on online questionnaires

Are the costs to be incurred well justified with regard to the subject and scope of the research?

In my opinion the costs are underestimated. Low budget of this proposal may constitute the most important obstacle.

Strengths of the proposal

Interesting idea with important interactions with international scientific community.

4. Weaknesses of the proposal

Weak development of the study idea – the hypotheses that are not adequately justified. Undeveloped potential impact of the study. Shallow understanding of the potential data structure. Low budget

Sandra Åhlén Mulio

Grant proposal review of “Behind the mask. Human abilities of face recognition during Covid-19 pandemic.”

1. Assessment of the scientific level of research or tasks to be performed

The outline of the project described is likely to result in publication. There is however some ambiguity in the current proposal that needs to be addressed before the start and finish of such a project. The scientific level behind the proposal is adequate in relation to research goals and tasks.

2. Assessment of the project’s innovative potential and impact on the advancement of the scientific field/discipline

The project has some elements of innovation and is adapted to be performed even in instances of future outbreaks which could otherwise put other projects to a grinding halt. The proposed project also has many interesting and important implications on future research and application in many fields and yet the beneficial outcome as stated in the proposal is somewhat limited. Authors are encouraged to think “bigger” for future proposals in order to truly convince the reader of the importance and possibilities of their research outcomes. Other areas applicants research results could have an impact on are for example psychology, anthropology, law and policy making and software development.

3. Assessment of the project feasibility

If one programmer is meant to design a program and all the features from scratch this might be very time consuming. Overall the time schedule seems tight if the project would run into problems at some stage, especially concerning recruiting 100 people from each country even through advertisement and remuneration might be an issue to take into consideration depending on the sample variance or randomness that is sought after. However, the project proposal in its concept is feasible and executable as a whole.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

The costs for advertisement are understated and also not specified, whether online ads, flyers, posters etc. Should printing of such material be carried in each country or do they need to be shipped?

Also, 37 000 PLN per month as a salary seems exaggerated even though software developers in countries such as the US are well paid. The justification for the scope and length of such software development are

unsatisfying. Perhaps using existing software and adding this new suggested database of pictures with covered face would be enough? If not, then why? On the other the PI salary is very low.

The cost for translation is not justified as it is calculated for 10 countries, when three of them already have English as an official language although depending on where in Canada you recruit, French might an option. If the survey is created in English, it only needs seven to eight translations, assuming applicants are targeting the main or most widespread language in the country, as Spain for example also have Basque.

5. Strengths of the proposal

The major strength of the proposal is the interesting topic which have important applications in many fields such as psychology, anthropology, law and jurisdiction, software development and areas mentioned by applicants.

The main ideas are outlined well and as a reader it is possible to understand the major steps in the project. Several hypothesizes exist that are formulated in a good way where it is easy to interpret what they do not prove if clarified (see comment in the next section about age).

Knowledge gap is identified and there is logical structure to the grant proposal. Tasks are clear and understandable. Analysis is clear in terms what statistical analysis is planned to be used in order to analyze the data. Language is also suitable for the general public as it does not jargon or technical terms that are difficult to understand.

The project proposal is well supported by scientific literature.

6. Weaknesses of the proposal

In general, the language of the grant proposal needs to be checked for grammar and style if applicants are not confident about their level of English proficiency. At the current level there are unfortunately many cases of incorrect grammar or inadequate word choice. Applicants are advised to make sure that the level of the written language is up to expected standards in future proposals. At the current state there is some ambiguity or parts which needs clarification in order to fully present the potential of proposed research. One such example is the hypothesizes regarding age where it is not clear whether the ability recognize faces or emotion decline with decreasing age or increasing age, as only “age” is stated. Readers of the grant proposal cannot be expected to guess even if it would be implied, it has to be made clear.

Further, it is stated that the project aims to identify groups which might have limited abilities to recognize faces and emotions with mask-covered faces and yet, you later mentioning excluding many groups which might have this problem. In this case applicants might need to reconsider phrasing of aims if these are not target groups.

A small note of gender bias, a software developer does not have to be a he. Applicants are advised to refrain from, when possible in future proposals, from using gender other than describing actual projects where gender is a central focal point or of interest of the study such as in applicants described hypothesizes. Missing in this proposal is a mention of confidentiality or anonymity for both participants and faces to be used in the database. Such information is important to provide to assure the reader that the applicants have considered everyone’s safety and integrity. Age group categories in methodology are not justified why chosen with current interval? Why not more intervals?

The proposal lacks clarification under which circumstances the project can take place, whether it is during the pandemic and lockdowns or when restrictions are not enforced? I point this out since it could help expanding the recruitment to advertisements at Universities since applicants intend to collaborate anyway with such institutions.

It is stated in costs that participants will be randomly chosen, how and when in the process? It also remains unclear if the aim is to have equal sample size of males, females and gender categories or what the representative sample would ideally be for the results to be representative.

In the summary of study outcomes there is statement of total inclusion as well as an out of place catchphrase belonging to a movement of unclear existence and with no previous mention. If applicants would have suggested organizing a workshop or seminar about the topic and relaying their results to the general public, this could indeed be positive. In other words, it is not bad, merely out of place and lacking context within the frame of the grant proposal.

Below I have listed spelling and grammar errors to further aide applicants:

Line 7, it sounds like you are saying that when in social interactions, the subject of face recognition is important, it sounds as if it is an important topic to discuss between people having a social interaction.

Line 7, “guides” word choice

Line 10, “still little is known” little is still known

Line 10, influences

Line 11, with the

Line 11, omit the first “to”

Line 12, the pandemic

Line 13, “The methods to be used for the study will be” The methods used in the study will comprise of...

Line 14, “additional using” swap places

Line 15, only one person, not people?

Line 15, participants’

Line 16, “check” word choice

Line 17, an issue

Line 19, see comments earlier about age

Line 19, societies or countries?

Line 19, age, increasing or decreasing age? Not clear.

Line 20, history

Line 20, could or will? It’s you prediction right?

Line 21, a positive

Line 27, peoples’

Line 27, the emotions

Line 28, affectivity means emotions, so emotions are strongly connected with emotions?

Line 30, “Nowadays” word choice

Line 31, novel, severare and acute..

Line 32, omit presenting

Line 33, a face mask

Line 34, the nose

Line 35, check sentence and “overcome” word choice

Line 36, the societies, maybe countries instead?

Line 38, the emotions

Line 39, they only cover...

Line 39, samples

Line 41, check beginning of line

Line 43, which groups pf people? Provide an example.

Line 46, Here it sounds like they are better recognizing people wearing masks.

Line 50,52 and 61, age, see previous comments about age.

Line 68, of improving

Line 73, at their faces

Line 76, exposure I assume? Not exposition?

Line 85, into

Line 11, in the form

Good luck in future endeavors,
Reviewer

1. Assessment of the scientific level of research or tasks to be performed

The project is well described and planned. It has a nice structure and easy to follow. The project has been written in due diligence, and it meets the criteria for a scientific proposal. The project has some elements of innovation which has not yet been studied thoroughly before which make it unique. The project results surely deserve to be published in a high ranking journal. The project identifies certain aspects which will lead to the scientific development of understanding human cognitive sciences. However, I am uncertain how the project will have direct insight on development of neurobiology or mental health therapy, as the PIs claimed. I could not find any further explanation in the project where they stated the impact of the project on neurobiology. I felt this might be the weakest link of the project which might need more explanation connecting the project's findings to psychosociology.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

The project definitely raises real-time issues currently associated with COVID-19 pandemic. There are certainly novel aspects in the project, and it should meet with interest in its application. The project has a greater applied aspect which makes this project innovative. I do see clear indication of the gap of knowledge and justification why your research is important. Proposal that focus on applied sciences is fast developing branch of biology what increase possibility of publication results of this research in journal as it was outlined in work plan.

3. Assessment of the project feasibility

The project duration (1 year) seems quite feasible considering the nature of the pandemic is really uncertain. This kind of relevant yet applicable research needs quick funding and for a shorter period of time. I think, the applicants did a good job in this aspect. However, I didn't find any mention of ethical issues being dealt when asking volunteers to participate. This aspect has to been mentioned as each country can have its own ethical requirements.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Budget is prepared carefully, and some costs are presented in detail and well justified. However, the only budget I didn't find appropriate is payment for the volunteers, which is 22 PLN. However, this money, according to me is not generous enough, at least in some countries where the cost of living is very high like USA or Japan. The converted money comes to \$5.5 in the USA, £4.5 in the UK. I feel this aspect should be consulted with respective countries to find optimum payment. I other cost that I felt was completely ignored was salary for a programmer. This cost is not listed in the budget or its exclusion is not well explained.

5. Strengths of the proposal

- Involving multiple countries with different genetic environment makes this study more applicable globally.
- The study addresses a very recent problem emerging in the western part of the world and also comparing it with countries where wearing a facial mask is not a very uncommon practise.
- Clearly defined aims and testable hypotheses.
- Well written project containing novel aspects.
- Clear links between tested hypotheses and planned experiments.
- The proposed methodology has been considered carefully.

- The potential risks well taken into consideration and planned accordingly. I also think compensating the volunteers with monetary help will aid in gathering enough data points.

6. Weaknesses of the proposal

- Line 83-84: I am still unsure how face recognition can help with “can help further develop mental health care system, especially clinical implications leading to development of COVID mental health therapy and drug development.” I am specially not convinced by the wording ‘drug development’. I find no clear or direct association with human ability of face detection to development of drug. No wonder, this study is really relevant in today’s pandemic situation but not necessarily the way the applicants mention in the third point. I would suggest avoiding such wording.

- Line 111 mentions “we will hire a programmer and provide him with all the details crucial for the study, that needs to be incorporated in the program.” However, in the budget justification (Line 269 onwards), I do not see any mention about hiring a programmer. Is the same person also the graphic designer or the price is included in equipment/program cost?

- Line 254: In the budget table, some values are separated by “,” and some by “.”

- The project does not highlight any scope for ‘negative’ results and its implication to broader cognitive science. My general question is “will negative face recognition have any consequences on overall results?”

- My final concern with this online method of face detection of unknown faces. In real life, people might not just associate known people with just seeing eyes. It has association with hair, height, body posture as well. One might be able to detect people by virtue of other aspects. So, the results of the project might not be a true reflection in real life scenario. For e.g. the project can have greater number of people unable to identify unknown face with masks so the result will be towards negative. However, in real life scenario, people might be able to identify more people due to associated factors as well which might push the result towards positive. This might create a bias in the actual outcome.

Generally speaking, I find this grant proposal interesting and applicable. The whole project is worth financing in my opinion, and authors are to be commended for undertaking such practical yet important topic in such a short time. With few modifications, I am sure the project will be worth testing.

Monika Ostap-Chęć

Proposed project concern human abilities of face recognition during Covid-19 pandemic. The main project aim is to investigate influence of age and sex on ability to identify faces and emotions during pandemic and whether people’s abilities to recognize faces and expressed emotions can improve when people are getting familiar with seeing mask-covered faces in their social interactions.

1. Assessment of the scientific level of research or tasks to be performed

Presented project has a valid scientific basis. Research hypotheses are constructed properly and supported by literature data. All needed part of the project proposal were included. The research methodology is presented in general way (a few more details could be included, see point 6). Some relevant factors were not taken into account when planning research (see point 6)

2. Assessment of the project’s innovative potential and impact on the advancement of the scientific field/discipline

Project touches on a very important subject especially in current difficult times. Social isolation that is necessary in the time of pandemic certainly contributes to the increasing difficulties in social interactions, what in turn can translate into psychological problems or difficulties in normal functioning. Wearing a mask

that cover more than half of face during shopping, at work, school, in public or service places presents another challenge from the psychological and sociological point of view. Analyzing which age and gender group have greater difficulties with faces and expressed emotions recognition may be crucial in identifying risk group (groups of people the most exposed to the negative effects of face-covering). It seems to be particularly important to check whether elderly people face the greatest problems with recognition. Certainly, results of this experiments by can be used by sociologists, psychologists and neurobiologists for further research.

3. Assessment of the project feasibility

The majority of tasks planned in the project should not cause problems in execution. Some doubts raise the terms of international cooperation. Particular Universities are not listed in the project. Some problems can make obtaining sufficient and representative group of participants. In order to make sure that the group is representative, it is necessary to ensure wide advertising of research in various environments in each country.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Presented budgeted costs are well justified. Planned costs do not take into account any additional funds for people from other Universities. What is more, planned payment for participants seems to be very symbolic amount, which may not encourage to participate in research, especially considering that amount of money in other countries.

5. Strengths of the proposal

The big advantage of planned research is that it touches on a very current topic. The concept of the research give the chance to identify groups that faces the greatest difficulties. Definitely, the strength of the planned research is finding out whether people get used to seeing other people with covered faces over time and whether recognition causes less and less problems with time. The results of such tests will indicate whether the problem will continue to grow or whether maybe it is only temporary. Another good point is that this kind of research could be performed relatively fast, so results can be used even during ongoing pandemic. For sure, the strength of proposal is international cooperation with many Universities around the world. Carrying out such tests on people of different nationalities enriches the results and strengthens inference. Research hypotheses are constructed properly and supported by previous research.

6. Weaknesses of the proposal

Overall, the designed concept is very interesting and worth exploring. However, in the presented project, I lacked reference to what emotions / states / difficulties are caused by the fact that people have difficulties to recognize each other or other's emotions. Performed test will answer whether a given age group or gender has greater / lesser difficulties with recognition, but still there is no information about consequences of that difficulties. My suggestion is to enrich the online test with the questionnaire about feelings that accompany people in such situation. It would be worth knowing if such situation evoke any negative feelings.

Another weak point I find is that test of recognition will be performed using photos of faces of people completely unknown to the participants. I have some doubts whether examining the level of face recognition in masks on unknown faces, especially through the screen of a monitor, gives reliable results. Generally, people who know each other recognize each other not only by their face but also by hairs, figure, clothes, glasses or movement. These factors should also be taken into account conducting such tests, otherwise, tests do not give results that reflect reality.

Another factor I would like to point out is origins of the participants. People living in small communities, in villages or small towns, usually know each other well and know most people that they meet in the street. The situation in large cities is completely different. Most urban people do not know the passers-by on a daily

basis. Origins of the participants is an important information and should be at least included in the questionnaire.

The research focuses on the difference in faces and emotions recognition according to the age. However, the proposed age ranges seems to be too broad. The 50+ age group includes both middle-aged people as well as the elderly and very elderly. It would be more beneficial to compare definitely narrower age ranges. The methodology is presented quite clearly. One thing that is missing, in my opinion, are details of the online tests themselves. There is no information how the preparative exercises and tests will look like. Will participants see a given face only once or they will learn the given face for a while? Will they have to match a face with mask to the correct face or by another method? Such information appears crucial in assessing the methodology.

To sum up, the project is very interesting, touches on current global problems and has a great potential. However, some additional factors and details still need to be considered.

Final project proposal

Title: Behind the mask. Human abilities to face recognition during the Covid-19 pandemic

Applicants: Debarati Bhattacharya, Sylwia Herman, Paulina Joško

Summary

Recognizing other people's faces and facial expressions are crucial aspects of social interactions. These abilities help us to uncover other's emotions, personalities, and gender. However, this recognition power is currently being challenged during the time of the Covid-19 pandemic with the majority of faces now being covered by masks. Unfortunately, little is known about the possible influence of other factors (such as sex or age) on the recognition of faces that are covered. With the intention to help to ease this global challenge, our study was designed to investigate the influence of age and sex on identifying covered faces and emotions during the pandemic. The methods used in the study will comprise of an online questionnaire followed by experimental tasks. In task A we will follow a Cambridge Face Memory Test (CFMT) modified by using additional images of people wearing a medical mask. In task B the participants' ability to identify emotions by looking at facial expressions on images will be tested. We also intend to verify if the long previous experience of interacting with people wearing masks improved the ability of facial recognition, the issue that wasn't examined so far. We predict that women will perform better than men in recognizing faces and emotions and this ability will decrease with increasing age in both task A and task B. While in countries with previous face-covering history people could perform better with recognizing covered faces and emotions. We believe that the outcomes of this study can have a positive impact not only during the Covid-19 pandemic but also provide new insight useful for neurological, psychological, and social sciences.

Keywords: facial recognition, emotions, face mask, pandemic, Covid-19

The scientific goal of the project

The ability to recognize people's faces and emotions they are expressing is a crucial skill in human communication and social interactions. Facial expressions are strongly connected with affectivity and pose a linkage between human bodies and their physical and social environment [Fox, 2015; Russell and Fernandez-Dols, 1997; Jingguang et al., 2010]. Presently, we are facing a worldwide pandemic caused by the novel "severe acute respiratory syndrome coronavirus 2" (Covid-19) that expresses itself with a wide variety of symptoms in patients [Beaupre et al., 2020]. Due to a droplet route of infection of the virus, a common way of preventing spreading the virus is using a face mask to cover the nose and mouth area [Ashtiani et al., 2020; Doung-Ngern et al., 2020]. It is now impossible to predict when it will be safe again to restrict from the mask-wearing regulations or when the pandemic will be overcome. As a result, people from countries all over the world are now facing a new challenge of interacting with others with their faces being covered by masks. There are some studies showing that the face-covering may affect the ability to recognize other people and emotions they are expressing [e.g. Esteves and Öhman, 1993; Kret and Fischer, 2018]. However, they only cover small study samples, therefore the analysis did not consider the possible influence of other factors, such as sex or age of the participants. The sudden emergence of the new interrupting factor on a global scale creates a unique opportunity for a deeper and more detailed study of the issue with a completely novel approach. Impaired facial and emotional recognition can significantly affect one's mental health [Szanto et al., 2012; Simcock et al., 2020]. Thus, it is important to understand in greater detail which groups of people can be more susceptible to having difficulties due to this now worldwide issue. In our study, we will attempt to identify those more vulnerable groups. Additionally, we intend to investigate if it is possible for people to adapt to this new condition and improve the ability to recognize faces and emotions when they are more familiar with seeing faces covered with masks, as it was not previously examined. The following hypotheses will be tested:

6. Females have a higher ability to recognize covered faces than males.
7. The ability to recognize covered faces declines with increasing age.
8. Females have a higher ability to recognize emotions expressed on covered faces than males.
9. The ability to recognize emotions expressed on covered faces declines with increasing age.
10. Societies with previous face-covering history are better with recognizing covered faces and emotions they are expressing.

Significance of the project

We formulated directional hypotheses, expecting women and younger people to possess higher recognizing abilities, based on the current state of the art. According to the primary caretaker hypothesis [Babchuk et al., 1985], the female role as a caretaker with children from the evolutionary perspective is being connected with the greater ability to correctly and rapidly recognize emotions. Whereas the biosocial model [Money and Ehrhardt, 1972] emphasizes the significance of the different emotional experiences and expectations that typically form females' socialization in their recognition advantage. Aging-related decline in cognitive functions, visual and sensory domains suggest that younger people will achieve better results in recognition tasks [Abbruzzese et al., 2019]. There are some studies indicating that women outperform men in recognizing emotions based on facial expressions [Montagne et al., 2005; Wingenbach et al., 2018], as well as recognizing faces [Lewin and Herlitz, 2002; Weirich et al., 2011]. What is more, other studies on facial and emotion recognition reveal that those abilities decrease with increasing age [Lamont et al., 2005; Sullivan et al., 2007; Mill et al., 2009; Abbruzzese et al., 2019]. However, in those studies, an additional factor of facial covering was not taken into consideration. Furthermore, those studies were conducted on the local scale, with smaller study samples, thus the general applicability of those results can be questionable.

There are no studies examining if people are capable of improving those abilities after adjusting to face-covering with masks over time. The suggested study can contribute to a better understanding of how sex, age, or long previous experience of interacting with people wearing masks can impact the human ability to recognize emotions and other people, by looking at their faces, covered and uncovered with the mask. It will help to identify groups of people who can have lower the above-mentioned capabilities while being fully healthy, and therefore, who may face greater difficulties in social interactions than others. Identifying vulnerable groups is crucial for providing early mental health care and support [Szanto et al., 2012; Simcock et al., 2020]. What is more, our research will be useful in assessing if longer exposure to interactions with masked people can facilitate face and emotion recognition. With the intent to conduct an international survey, our study will cover the data collected across the world, giving our project a unique global approach and significance.

To sum up our study outcomes:

- can show the need for improving communication during the pandemic time and highlights the potential reason for soft skill deterioration e.g. in the job environment.
- can help to extend the mental health care and mental health therapy on people with lower ability to recognize covered faces and emotions expressed on them.
- provide new insight into neurobiology, anthropology, psychology, sociology, and psychosociology due to additional details about differences between sexes and age groups in covered face-recognition.
- would contribute to criminology, forensic anthropology, and forensic psychology development. The crime witnesses of a given sex or age group can appear to be more trustworthy in the face-recognition of masked criminals.
- can contribute to the development of medical mask design and policymaking e.g. ordinance of using masks made from a transparent material.
- can be useful in clinical diagnosis and therapy development of several neuropsychiatric disorders.

Concept

We intend to conduct our study in 10 countries: the USA, Canada, United Kingdom, Spain, Italy, Germany, Poland, China, South Korea, Japan. We selected this list considering high Covid-19 incidences (according to WHO data) and the level of country development (common access to the internet is necessary). We included specific Asian countries, due to their long previous experience (of at least 3 years) of wearing medical face masks by residents, e.g. Japan [Burgess and Horii, 2012]. We decided to focus the comparison on the countries with a history of using medical masks, as this is the type of covering used now during the pandemic, and therefore, the covered area of a face is very similar, and the rest of a head remains uncovered. However, it would be interesting if future studies would compare our results with Muslim countries, where the history of women covering their faces and heads is much longer. We plan to collaborate with other universities in those 10 countries to help advertise information about our survey and collect data. In our project, images presented to volunteers will focus only on the faces, because face plays a key role in the recognition of others and is proven to be the most important factor that people consider while identifying others and their emotions [Fox, 2015; Russell and Fernandez-Dols, 1997]. Therefore, we believe our results will be reliable and applicable in real-life situations. Nonetheless, future studies could incorporate additional factors (e.g. body posture, voice) in the analysis. We plan to compare obtained results based on the gender of volunteers, their

age, or long previous experience with social interactions with other people wearing medical face masks. We would like to carry out our research on two sexes (men and women) and three age groups (A. 21-34-young adult, B. 35-50- mature adult, C. 50+-old adult). We have chosen these age groups based on previous studies [Abbruzzese et al., 2019]. Nevertheless, the youngest participants we consider are twenty-one-year-old people for the sake of necessity of getting the parent permission in some of the selected countries (e.g. the USA) to participate in the remuneration payment which can be problematic to verify. We schedule the project duration for 1 year.

Research Goals

To address the issue of the influence of age and sex on the ability to identify faces and emotions during a pandemic.

To investigate whether people's abilities to recognize faces and expressed emotions can improve when they are getting familiar with seeing mask-covered faces in their social interactions.

Work-plan

8. Developing a program that will display the questionnaire, preparatory task, and experimental task to participants in the form of an online survey. For this purpose, we will hire a programmer and provide all the details crucial for the study, that needs to be incorporated in the program. Design can be based on already existing software and be adjusted for the specifics of our project.
9. Preparing our own database comprised of a wide range of digital images of human faces to display in tasks. To simplify this step, we could use already existing image databases but modify them, by covering the mouth and nose with a face mask. For this purpose, a professional graphic designer will be hired.
10. Finding potential collaborators in pandemic affected countries.
11. Advertising through local Govt pages, social media, health workers, NGOs, Religious institutions using leaflets and posters
12. Collection of data – conducting the online survey.
13. Data analysis.
14. Publishing results of our study in a form of scientific articles.

Risk analysis

Establishing new collaborations with universities from other countries can pose a challenge at the beginning of the project. Nevertheless, we believe that in each country we will be able to find collaborators, as the Covid-19 pandemic is a relevant and important issue on a global scale. Another difficulty we may encounter would be to not gather enough volunteers in each country. To prevent that, we will invest in advertising our study through various sources, as well as encourage people to participate in the survey, by offering relatively high cash remuneration. What is more, we will invest in translating the survey to languages other than English (depending on the native language of the country considered), to include non-English speaking volunteers. Finally, there is a possibility of no differences being detected in our study, but we estimate it to be a low risk, considering the current knowledge in the topic. Nevertheless, such results could still be published, as they will provide valid insight into the subject.

Methodology

For our study, we will question a group of volunteers from a range of countries affected by Covid-19. We will design an online survey consisting of a short questionnaire, preparative exercises, and a final experimental task. In the beginning, the program will display a communicate, that the participation is voluntary, and that we will ensure the confidentiality of each person. Below, we will ask for consent to participate in the survey. The participant will have an opportunity to leave a contact email address, if they wish to be considered for the remuneration and informed about their results of individual tasks – everyone can proceed further without leaving the contact information and email addresses won't be connected to the participants' answers to ensure the anonymity. A short questionnaire will provide us with the necessary information about a volunteer (sex, age, country). We will also incorporate questions that enable us to exclude people with other disorders that can influence their performance of the test (such as visual imparity, autism, congenital prosopagnosia symptoms, psychiatric illness, alcohol or drug abuse). The preparative exercise is a necessary step to familiarize the volunteers with the computer program used in the study and task rules. Following the example of the Cambridge Face Memory Test [CFMT; Duchaine and Nakayama, 2006], in the preparation for the task A, volunteers will be presented with a cartoon face for 3 seconds and then asked to

identify this cartoon face from a group of three different cartoon faces shown. This would be repeated 5 times. While for the preparation to task B, the participant will see a series of faces without a mask, each expressing different emotions. A single image will be shown at the screen for 3 seconds and then volunteers will choose which emotion was the face expressing from the list of all 7 possibilities displayed. This will be followed by the experimental task. Each volunteer will prepare for and perform task A and then task B. Both tasks rely on displaying various face images to the participant, examples of pictures that could be used for that purpose are presented in Fig. 1. and Fig. 2. Faces on all the images should be orientated in the same position and facing the same direction to exclude some misleading by other distractions. The overall time for completing the survey should not exceed one hour. The program will randomly choose participants (100 from each country, based on emails provided) that will be compensated for their effort with 50.00 PLN per person. The survey will be prepared in English but, if necessary, can be translated.



Fig.1. Exemplary pictures of faces with and without masks, that could be used in our study. Provided from: <https://philadelphia.cbslocal.com/wp-content/uploads/sites/15116066/2020/08/face-mask-study.jpg>; modified.

Task

description

Task A. To test hypotheses 1 and 2 we will prepare a task, in which participants are going to recognize faces from images presented to them. We will design our task based on the standard CFMT; [Duchaine and Nakayama, 2006], which is suitable for comparing age groups and genders [Cho et al., 2015]. However, in our study, we will modify standard CFMT, by adding images of faces with masks covering the mouth and nose area. Immediately after the preparation for task A, the participant will start an experimental task A, in which a picture of a target face will be displayed for 3 seconds. Subsequently, a set of 3 different pictures with covered faces will appear on the screen, including the target face, and the volunteer will be asked to identify it. Another set of 3 pictures would be presented afterward with the same target face to identify. Then the new target face will be shown on the screen and the procedure will continue as previously.

Task B. To test hypotheses 3 and 4 we will design a task, in which participants are going to identify emotions expressed on faces presented to them on images. We will prepare a database of face images showing all basic expressions of emotion (happy, sad, angry, fearful, disgusted, surprised) and neutral expression, with an addition of a mask covering the area of mouth and nose. Experimental task B will follow the same principle as the preparation for it, but the faces that are shown to the participant will be covered by the medical mask.

To test hypothesis 5, we will compare the results obtained in 3 selected Asian countries to the remaining 7 countries.



Fig.2. Exemplary pictures of covered faces expressing the basic emotions, that could be used in our study. Provided from: Dailey et al. 2020; modified.

Results analysis

We will receive binary data (correct/incorrect answer). Thus, we are going to use logistic regression with the following predictors: age (as a continuous variable), sex, and the country. For all tests, the R program (version 4.0.2) will be used.

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Budget of the project

No	ITEM	Funds for each budget year (PLN)	
		2021	TOTAL
1	Direct cost including	186 300	186 300
	-Salaries and Scholarships	54 000	54 000
	-Equipment and Software	7 000	7 000
	-Other direct costs	125 300	125 300
2	Indirect costs (20% of direct costs)	37 260	37 260
	Total costs (1+2)	223 560	223 560

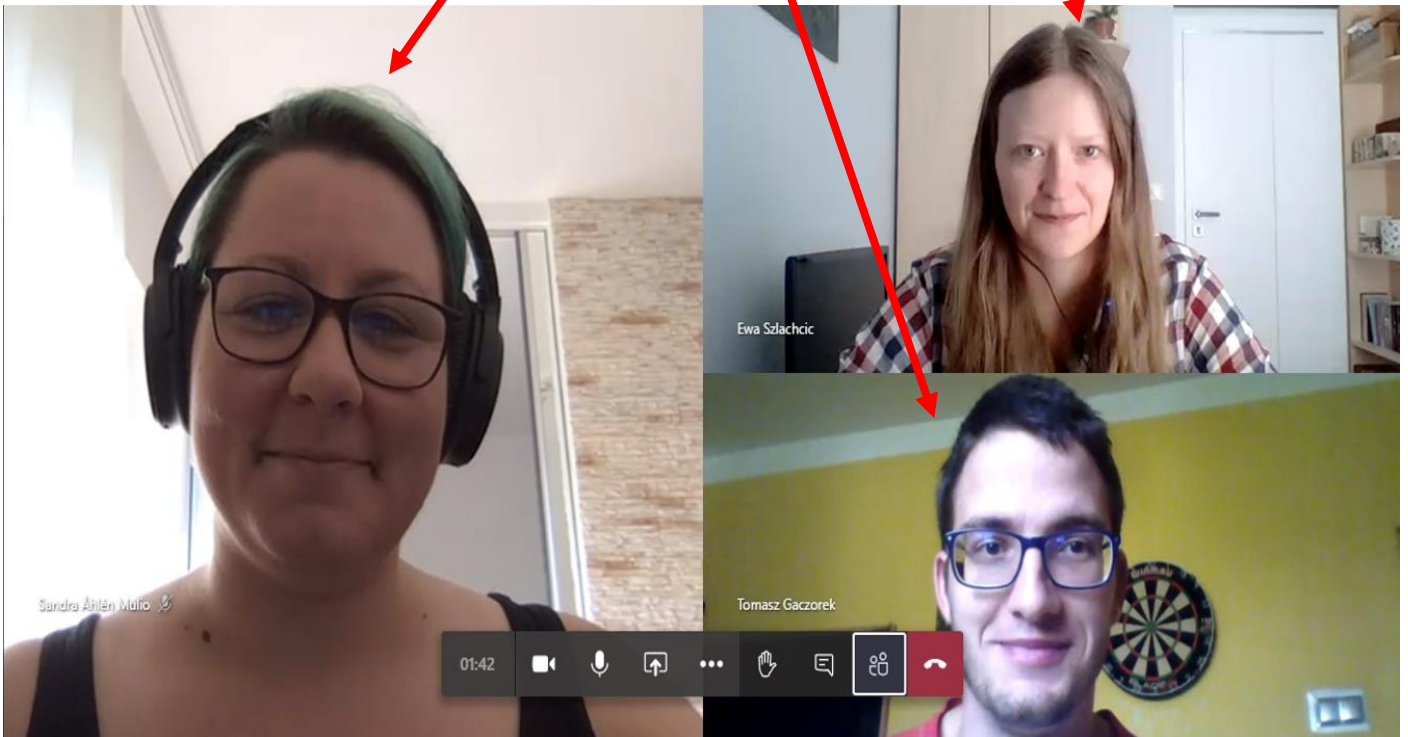
Breakdown of project costs including justification and relevance for the tasks in the project

1. Salaries and scholarships: 54 000 PLN
 - Salaries for PIs: 1500 PLN per PI per month. Total cost for three PIs, for a year: $1\,500 \times 3 \times 12 = 54\,000$ PLN.
Main tasks of PI: planning, online survey preparing, the database of face images preparation, regular consultations with the programmer and professional graphic designer, statistical analyzes, publication writing, other essential work (documentation, organization, etc.).
2. Equipment and software: 7 000 PLN
 - Designing the software program (that amount to hiring a programmer): total cost 5 000 PLN. This covers a remuneration for a programmer who will be hired. The main task - to make necessary changes in an already existing program based on our experimental demands. Since the task requires only adjusting software to our needs, we estimate that this job can be completed in two weeks' time. This constitutes to 10 working days, eight hours each.
 - Preparing a database of pictures: total cost 2 000 PLN. This covers a remuneration for a professional graphic designer who will be hired to modify the pictures according to our needs and create a database to store images. The total cost is estimated according to the time needed to make modifications and create a database of face images. The graphic will be hired for 1 week. This constitutes to 5 working days, eight hours each.
3. Other direct costs: 125 300 PLN
 - Remuneration for the volunteers: total cost 50 000 PLN. Payment per person for participation in the survey: 50.00 PLN. We will pay 100 randomly selected participants from each of the 10 pandemic affected countries. Total cost: $50 \times 100 \times 10 = 50\,000$ PLN. The money transfer will be done through online services like PayPal to the respective participants.
 - Payment for translating the survey and printed advertisement to languages other than English in non-English-speaking countries: total cost 1 400 PLN. We will fund 1 institute per country, a total of 7 institutes. Each institute will be paid 200 PLN to translate the questionnaire and provided templates of the leaflets and posters. Total cost: $200 \times 7 = 1\,400$ PLN.
 - Printed advertisement: total cost 65 000 PLN. We will cooperate with health workers, NGOs, and religious institutions in order to distribute printed advertisements, such as leaflets and posters, in all 10 countries. We will provide digital templates for the leaflets and posters to each country (send as pdf files via an email) and cover the costs of printing them at each location. The estimated cost of printing according to the current worldwide rate is 4 PLN per printout. We want each country to distribute 1 000 leaflets, therefore the cost will be: $4 \times 1\,000 \times 10 = 40\,000$. Additionally, we want each country to print 50 posters, the cost of a single poster is estimated to be: 50 PLN. Therefore, the total cost for printing posters will be: $50 \times 50 \times 10 = 25\,000$ PLN.
 - Online advertisement: total cost 900 PLN. For ads on Facebook, the cost for each ad is ~30 PLN per day. For a month of advertisement, the total cost will be: $30 \times 30 = 900$ PLN
 - Publication cost: in Health Journals the amount stands at 8 000 PLN (based on the current rates).

Project 3:

The characteristics and origin of symmetry detection in rhesus macaques (*Macaca mulatta*)

Sandra Áhlén Mulio, Tomasz Gaczorek & Ewa Szlachcic



Draft project proposal

The mechanisms and origin of symmetry detection

Tomasz Gaczorek, Ewa Szlachcic and Sandra Åhlén Mulio

Summary

The sense of symmetry is inherently related to humanity as it plays a central role in our aesthetic preferences. Previous research has provided evidence that non-human animals can also detect it. However, little is still known about the actual pattern of recognition and its nature. Here, we propose three experiments conducted on rhesus macaques (*Macaca mulatta*) that can potentially shed some light on the underlying mechanisms. Our first two experiments will test suggested detection advantage of vertical bilateral symmetry (VBS) commonly met in a natural environment and animals' body structure. Finally, we will assess whether the sense of symmetry is innate or unconsciously learnt based on surrounding texture. The outcomes of the proposed study will hopefully contribute to better understanding of the cognitive processes as well as factors driving sexual attractiveness.

1. Scientific goal of the project

Symmetry is common in nature and became the object of research concerning the phenomena of symmetry detection and speculation about its mechanisms (Thomas et al., 1992). There is growing evidence that organisms are sensitive to symmetries (Enquist & Johnstone, 1997). Many studies of symmetry preferences have focused on attractiveness. Female preferences for male symmetry have been found in humans (e.g. Jones et al., 2001; Scheib et al., 1999), zebra finches (Swaddle & Cuthill, 1994), and other species (Møller & Thornhill, 1998). However, there is also research showing preferences for symmetry and ability to detect it in non-mating contexts in different animals, for instance bees (Giurfa et al. 1996; Lehrer, 1999), spiders (White & Kemp, 2020), pigeons (Delius & Habers, 1978; Delius & Nowak, 1982), dolphins (Thomas et al., 1992).

There are several alternative explanations for the occurrence of the similar symmetry preferences in nature. Firstly, preference evolved for adaptive reasons connected with mate choice (Johnstone, 1994) as the degree of symmetry provides information about the quality of signaler (Little & Jones, 2006). Secondly, symmetry preferences can arise as a by-product of common properties of biological recognition systems (Enquist & Arak, 1994) like e.g. generalization (Enquist & Johnstone, 1997). Thirdly, symmetry preferences can arise as a by-product of the need to recognize objects regardless of their position and orientation in the field of view (Enquist & Arak, 1994).

Bilateral symmetry is the most common symmetry observed in animals and is highly conserved during the evolution of animals (Li et al., 2020). It is easier to recognize it when the axis of symmetry is vertical in relation to the retina than the symmetry of other orientations (Herbert & Humphrey, 1996). Bornstein et al. (1981) stated that the ability to recognize vertical symmetry in humans may be innate or very easy to learn. However, the origin and mechanisms of the symmetry detection remain unclear.

Our research questions involve different characteristics of the symmetry sensing.

The first question concentrates on the detection mechanisms. We investigate whether the pattern of detection depends on what is frequently met in nature (including own body shape). We expect that macaques will more easily detect vertical bilateral symmetry (Fig. 1b) than any other. To test it we will verify two hypotheses:

I. Macaques are better in recognizing shapes with one symmetry axis than asymmetrical ones or the shapes with more axes.

II. Macaques' ability to distinguish between symmetrical and asymmetrical objects decreases with increased deviation from the vertical position.

The other question is related with the emergence of symmetry detection. We want to examine whether it is an effect of conscientious learning based on the surrounding environment or an innate evolutionary response. The learning nature will be supported if:

III. Both groups of macaques (raised in the environment fulfilled with symmetrical or asymmetrical objects) will show similar attachment to the environment of the initial type.

2. Justification and significance of the project

Animals perceive their world differently by being affected by varying stimuli. Many vertebrates such as primates and humans rely heavily on visual cues to receive information about food, potential threats, conspecifics and when choosing a mate. Understanding the underlying evolutionary mechanics of how such information is gathered is essential for our knowledge about their ecology and behaviour. Our project will be the first one to investigate whether the ability to detect and preference for bilateral symmetry is an innate state of the rhesus macaques (*Macaca mulatta*). Furthermore, symmetry is an abstract visual pattern, i.e. because symmetry relies on relation of objects together rather than the properties of the objects themselves (Thomas et al., 1992) our project would identify not only the degree of taught perception of an abstract concept, but also an innate ability to perceive it regardless of training and thus, to some degree, understand abstract concepts. Understanding the nature and utilization of bilateral symmetry in rhesus macaques will provide valuable insights into the evolution of a visual aid and how it compares between old world monkeys and humans.

Our findings will:

- Provide new insights into human and animal evolution by understanding how new traits can be created as by-products of other necessary features of survival. As suggested by (Enquist & Arak, 1994), a preference for symmetry as a phenomenon can sometimes only be discovered if the objects of the preference appear in existence. Thus, detection and utilization of bilateral symmetry prompt science to further investigate such instances.
- Further provide an understanding of how rhesus macaques and potentially other animals use or do not use symmetry when choosing a mating partner. It has been shown in humans that bilateral symmetry is correlated to attractiveness and health, and as such, act as important visual cue input when choosing a partner. In insects, symmetrical ornaments that are more symmetrical have been proven to be more attractive. Therefore, this could have further implications in helping wild animals in captivity with successful breeding such as in many conservation breeding programs in zoological gardens since individual specimens options are limited.
- Provide a foundation for future studies on other vertebrates and the potential improvement of aforementioned captivity breeding programmes for endangered species.

3. Concept and work plan

The general work plan of the project will include the following tasks:

Task 1: Internship in the institution conducting research on rhesus macaques.

Task 2: Preparing facilities and maintaining rhesus macaques. After acquiring necessary supplies (cages, food, etc.) we will begin maintenance of rhesus macaques.

Task 3: Measuring the rhesus monkey's ability to detect different orders of symmetry (Experiment I). This task will provide information for testing hypothesis I.

Task 4: Analysing the results from the first experiment. This step is indispensable for starting the second experiment.

Task 5: Investigating how the orientation of an object affects detection of its symmetry (Experiment II). This task will provide information for testing hypothesis II.

Task 6: Conducting the third experiment which is divided into two parts: rearing animals in three environments, fulfilled with symmetrical, asymmetrical or mixed objects for one year after which observations of time spent in the three environments by free choice will be recorded. This task will provide information for testing hypothesis III.

Task 7: Analysing results and preparing publications.

Projected time of the tasks is visualised in table 1.

Table 1. The timetable of the project.

Calendar date (month-year)	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23	May-23	Jun-23	Jul-23	Aug-23	Sep-23	Oct-23	Nov-23	Dec-23			
Tasks / Month in order	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36			
1. internship																																							
2. rearing monkeys																																							
3. conducting experiment I																																							
4. analysing results																																							
5. conducting experiment II																																							
6. conducting experiment III																																							
7. analysing results																																							

Our project involves little risk as we will follow established guidelines and protocols of animal testing in regards of animal health and care to avoid injuries and disease of animals and staff as by the EU directive 2010/63/EU. Any facilities or tools used in experiments will be designed following the same guidelines.

4. Research methodology

All subsequent experiments will be performed on rhesus macaques (*Macaca mulatta*, Fig. 1a) raised and kept in captivity. The model organism was chosen based on previous research showing its abilities to detect symmetrical objects. However, the sensitivity of detection varied significantly between experimental designs and presented objects (Waitt & Little, 2006; Sasaki et al., 2005).

All studies presented below are planned to be carried out on 20 1-year old macaques per each experimental group. In total: I (80 individuals), II (60 individuals) and III (60 individuals). It would require standard equipment for studies made on non-human primates (as described in EU recommendations - 2007/526/WE).

I. First experiment focuses on the detection of different orders of symmetry. Macaques will be exposed to the buttons of distinctive shape, each having a variant number of symmetry axes (0,1,2 or 3) from which at least one is vertical. Depending on the experimental group, monkeys are going to be granted with food after pressing a button with desired symmetry order. Both, the buttons' position and its shape will change each day (repetitions allowed) but the representation of different symmetry orders within a set will remain constant. After 2 weeks of learning (the timespan based on Bertrand, 1967), monkeys will be exposed to shapes of buttons never seen before but the food award will still be correlated with a given number of symmetry axes. Then, the time until they reach food and the sequence of buttons pressed will be recorded. (Fig. 2a)

II. The second experiment is going to check how the orientation of an object affects detection of its symmetry. Based on the first study, only one well-detected, symmetrical shape will be used further. Then, its two modified asymmetric counterparts will be created with an increased or decreased total figure's area by approximately 5%. The modifying value is chosen to be significantly higher than asymmetry commonly met in nature but simultaneously not changing the figure's shape drastically. Macaques will be initially trained for 2 weeks to recognize given symmetrical shape among other, slightly differentiated figures. The protocol will be similar to the one for the first experiment. However, to avoid an attachment to any figure position their orientation will change randomly during the learning stage. After training, monkeys will be exposed to 3 buttons of shapes corresponding to the initial symmetrical figure and two modified counterparts. Depending on the experimental group shapes will be rotated by either 0, 45 or 90 degrees in relation to the initial vertical position. Then, the time until they reach food and the sequence of buttons pressed will be recorded. (Fig. 2b)

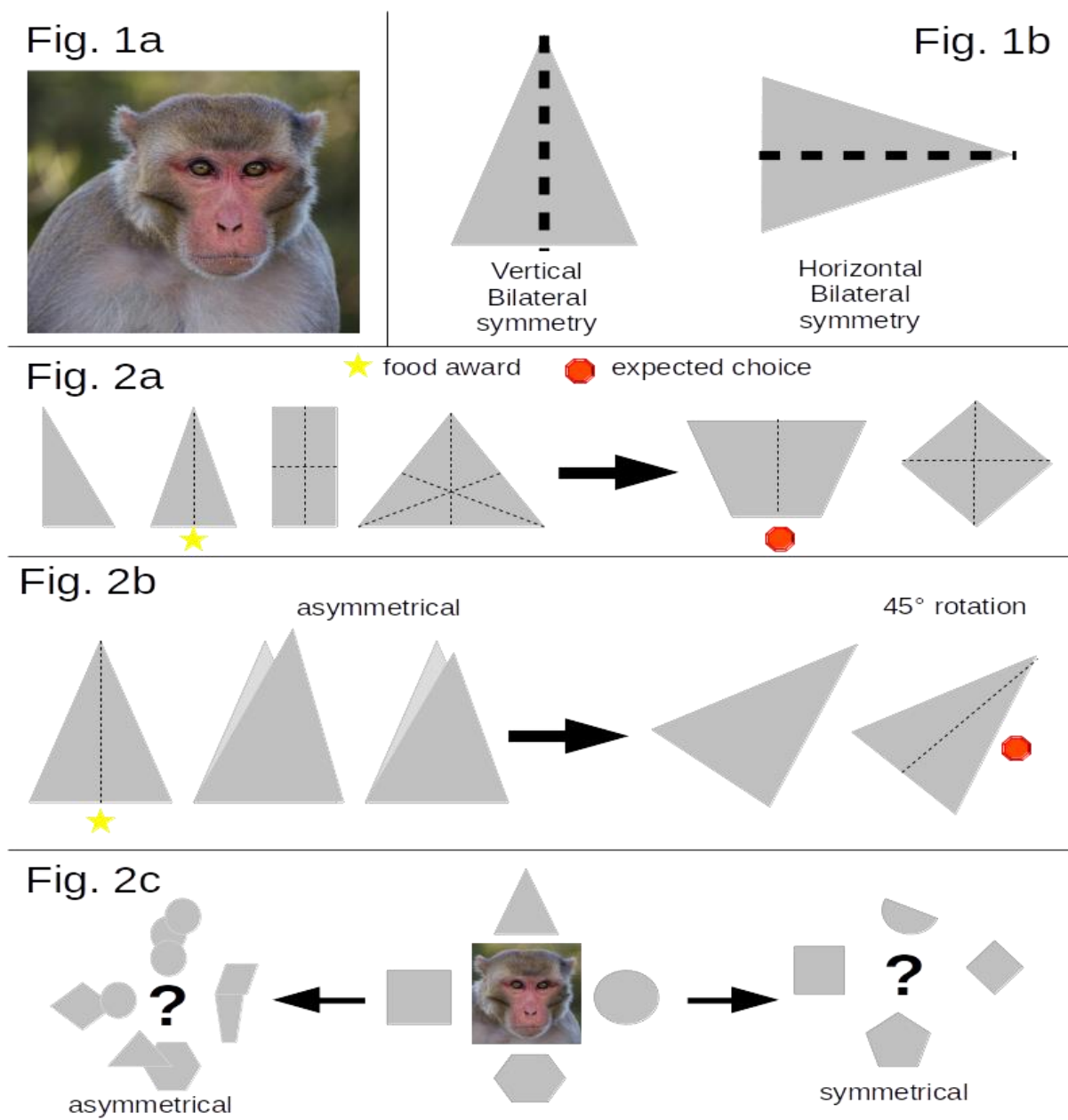


Figure 1-2c. Model organism and general scheme of experiments.

III. The last experiment will examine whether the nature of symmetry detection is exclusively related to conscientious learning based on the surrounding, highly symmetrical environment or it is an innate evolutionary response. To test it, 3 groups of macaques will be kept individually (with mothers) for 1 year after birth in the environments created of symmetrical, asymmetrical or mixed objects. After which they will

be given an opportunity to choose between a symmetrical or asymmetrical milieu. Importantly, the objects within the target environments will be different from those present in the initial one. The observations will be conducted by measuring the time spent in each target environment during the first week. Note that all environmental characteristics (other than objects' shape) will be constant throughout the experiment. (Fig. 2c)

Observations gathered in each experiment will be analysed in the R software (R Core Team) with the use of standard statistical tests as t-test, chi-square test and analysis of variance (ANOVA).

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Table with budget of the project

No.	Item	Funds for each budget year (pln)			
		2021	2022	2023	Total
1	Direct costs, including:	8 584 245	2 133 000	1 863 000	12 580 245
	- Salaries and scholarships	220 000	220 000	220 000	660 000
	- Equipment and software	956 500	540 000	240 000	1 736 500
	- Other direct costs	7 407 745	1 373 000	1 403 000	10 183 745
2	Indirect costs (20%)	1 048 354	1 048 354	1 048 354	3 145 062
	Total costs (1+2)	9 632 599	3 181 354	2 911 354	15 725 307

Breakdown of project costs including justification and relevance for the tasks in the project

1. Investigator/staff costs

Principal Investigators salaries: 540 000 PLN (3 PIs x 36 months x 5000 PLN)

Scope of work: planning, coordination, supervision of research, teaching master students, participation in gathering data, writing manuscripts.

Project-related remuneration period: 36 months

Salary cost for one PI: 5000 PLN/month

Technical assistant salary: 72 000 PLN

Scope of work: daily maintenance (e.g. feeding, cleaning), assistance during experiments, analysing videos

Project-related remuneration period: 36 months

Salary cost: 2000 PLN/month

Master students scholarships: 48 000 PLN (2 MSc x 12 months x 2000 PLN)

Scope of work: collecting of data in two experiments

Project-related remuneration period: 12 months

Scholarship for one MSc: 2000 PLN/month

2. Equipment/software

Renting zoo's facilities for the purpose of animals maintenance: 900 000 PLN (10 rooms x 5000 PLN/month x 1,5 year)

Creation of artificial enclosures including hall renting, maintenance and adjustment: 820 000 PLN (100 000 PLN initially + 20 000 PLN per month x 3 years)

Image recording set: 4 cameras, computer with software: 6000 PLN

Laptops: 9000 PLN (amount: 3)

Hard drives: 1500 PLN (amount: 3)

3. Other direct costs

Materials/Animals

Import and breeding monkeys: 13 800-38,640 PLN each, expected - 6 000 000 PLN in total

Cost of maintaining rhesus macaques per year: 1 372 000 PLN. Costs for three years: 4 117 200 PLN

Office supplies including printer cartridge: 1 000 PLN per year. Costs for three years: 3 000 PLN

Conferences and business trips

Costs of attending conferences of three PIs: 30 000 PLN

Internship in institution which conducts experiments on rhesus macaques: 34 745 PLN

(Flights (return tickets to US) 9 545 PLN, lodging 25 200 PLN for 1 month for 3 PIs)

Other costs

Costs of publishing (including open access): 15 000 PLN

Reviews

Prof. dr hab. Jan Kozłowski

The title is too general; “in rhesus macaques” should be added.

Abstract is too short for 15 000 000 PLN project. Not all aspects of the problem are covered or are covered but not detailed enough. General sentence “Our first two experiments will test suggested detection advantage of vertical bilateral symmetry (VBS) commonly met in a natural environment and animals’ body structure” serves as the description of two interesting experiments. This is not enough

The project. It is not my job to concentrate on details; I was asked for general overview of all four projects. I like the experiment design: in my opinion, the experiments are innovative and can improve our knowledge of the field. However, the project is not realistic. After reading summary, I thought that experiments will be based on ZOO animals, may be animals from a few ZOO’s. Of course a sample would not be so uniform and more advanced statistics would be required because of all confound factors. Building large captive group of primates would cost a lot (this is the main component of all costs, likely too low anyway) and would require a lot of time: probably three years may be not enough to get all permissions, assuming that such permissions will be granted at all. I do not think that any funding agency would pay so much for these experiments.

Dr hab. Łukasz Michalczyk,

1. Assessment of the scientific level of research or tasks to be performed:

The project is ambitious and the majority of the research tasks are planned correctly. Thus, in my opinion, it can be ranked high in terms of the scientific level.

2. Assessment of the project’s innovative potential and impact on the advancement of the scientific field/discipline:

The project is interesting and addresses important aspects of mammal behavior that significantly affect our life. If the hypotheses are properly tested, the results are likely to be published in top journals.

3. Assessment of the project feasibility:

Weak.

The team has demonstrated no evidence of their competence and experience required to carry out such an ambitious and difficult study. If the project requires a thorough training of the PIs in order to complete the study, while there is no evidence that an appropriate institution is willing to provide the required training and assistance (in fact, not even the name of the institution is provided), the project cannot be assessed as feasible.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?:

No.

The budget is too large in relation to the advances the project may provide.

5. Strengths of the proposal:

- An interesting and important topic.
- Properly formulated hypotheses and their predictions.
- Sound experimental design of the majority of parts of the project.
- The project is written well.
- An inclusion of a figure illustrating the ideas behind the project (however, the figure could be smaller, which would save some space).

6. Weaknesses of the proposal:

- It is not clear how many shapes will be provided in order to test hypothesis I. This is important as the range of choice may strongly affect the results of the experiment.
- Is the set up in experiment III appropriate for a healthy development of macaques? Given that these are highly social animals, keeping a single mother with a child without contact with other monkeys is likely to affect the results of the project.

- Is it possible to create an environment without symmetry? What about mothers that their offspring will observe?
- The length of the exposure to different types of environment in experiment III is not justified (why a year?).
- An important ethical issue: it has not been specified what will happen with the monkeys after the experiment.

Arpan Kumar Basak

1. Assessment of the scientific level of research or tasks to be performed

The scientific research proposed is beyond my field of interest. However, I could keep up with the text as the description of the project is fairly structured. If I understand correctly, the project aims to investigate whether the pattern of detection depends on natural observations or an innate evolutionary response. Overall the project seems to be conducive as the choice of model organism or subject as rhesus macaques is justified. Hypothesis set to test the questions seems to be aligned. But the impact of the outcome of the experiment, when alternative is true is not clear.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

It is important to investigate more into the science of symmetry and underlying mechanism of their perception. This could potentially provide us a validation on female preferences on males as applicants have stated along with nature of the trait alone.

3. Assessment of the project feasibility

The project is scheduled for 3 years, which is quite feasible. In principle, the last 7 months can be squeezed to 4 or 5 months and 2 months or so could be taken into accounting at the beginning of the experiment I where applicants will need time to assess the tools used in the experiment and perhaps spare 3 subjects to conduct the feasibility of the experiment.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Yes, the budget breakdown is well structured. Stationaries and other amenities, all necessities are stated and well defined. However, I noticed overestimation of the samples. As I couldn't find the justification behind choosing 20 individuals, especially when there could be a possibility that the results of 1st experiments show alternative expectation. I believe applicants can tackle this problem by conducting a pilot experiment.

5. Strengths of the proposal

It is true that sense of symmetry in a way plays important role in recognition of micro and macro objects. This coherent with the nature of distinguishing colours. Therefore, it will be interesting to examine the underlying neurological phenomenon and associated molecular mechanisms that coordinate in symmetry identification along with other visual perceptions.

The scientific goal of the project shows fair amount of literature behind the idea of the project. I appreciate authors effort in going through all the articles carefully before constructing this interesting yet expensive project. The list of research questions that authors ask (though it was difficult to find the questions), follows testable hypothesis.

Justification of the stated project that claims bigger than actual, is difficult. Here, applicants have supported their argument by stating that, apart from all other "known factors" or traits of "symmetry" stands out.

6. Weaknesses of the proposal

126-127 “All studies presented below are planned to be carried out on 20 1-year old macaques per each experimental group. In total: I (80 individuals), II (60 individuals) and III (60 individuals).”

I was not sure if I found the basis of choosing the individuals. Maybe it is me, but I couldn't find the information about the sex of the chosen animal system to be conducted in the study.

It was difficult for me to follow the experiment design from the figures. Someone has to take more than designated hours to read through the text and follow the figure back and forth. Applicants should make clear diagrammatic representation of the experimental design to make it more informative.

I presume the object (in applicant's case the button with manipulatable parameters) will be presented to the subject against which they will be rewarded. I hope the colour of the button will be constant throughout, otherwise it could be a colour of the button not entirely the shape that draw their attention. This brings me to the question, where was the cost justification of designing special button was stated.

Here, authors may consider a repeated measure with legitimate number of individuals before moving to other experiments. Also, my recommendation is to check the experiments if that is the ONLY way to test their hypothesis.

112-114 “Our project involves little risk as we will follow established guidelines and protocols of animal testing in regards of animal health and care to avoid injuries and disease of animals and staff as by the EU directive 2010/63/EU. Any facilities or tools used in experiments will be designed following the same guidelines.”

I was surprised that way applicant plans to conduct the experiments (rather the entire project) without certified veterinarian. Therefore, I assume one of the PI is a veterinarian.

The risk assessments are pushed towards the EU guidelines, which is fair. But I am sure authors have considered in their mind that if during the experiments there is a compromising situation with one or more individuals, they can come up with a contingency plan. However, I was not able to find such explanation in the risk assessment part nor a veterinarian at rescue.

I missed the part where authors have mentioned that there could be an underlying molecular mechanism that could assist in understanding the subject's ability to recognize symmetry. I am not sure if tools used neurobiology will be of any assist.

Lastly, even though the claims are high for basic science research, this could be a foundation for finding the mechanism of symmetrical partners (as cited Jones et al., 2001; Scheib et al., 1999), and/or understand the root of the trait of symmetry. I would encourage applicants to provide preliminary evidences (at least from a small pilot study on few samples or genetic data) and a structured short version that explains the project as clearly as possible. Currently, based on the description of the project, it is a little RISKY to fund 15 million PLN just to excavate the mechanisms and origin of symmetry detection. However, if the applicants address the issues as stated above, it will be really interesting to find the outcome of the research question.

Debarati Bhattacharya

The research tasks look interesting and achievable. The knowledge about food as a reward system and learning behavior is known to all of us from life sciences but the idea of involving symmetry changes the whole game. So, the potential of this project looks promising.

Well as it is written it is the first of its kind research so we must wait for the deliverables to see how it really matches the expectations of the authors and contribution to the field of animal research. If the expectations

turn true, then it will be something to look up to and will open a possible ocean of ideas to carry out further research in this field of symmetry.

Well as per the feasibility of the project it can be done, I mean the experimental task, but the experimental subject is proving to –“cost an arm and a leg” for the PI and host institute.

Well as the budget is concerned, I personally believe that no good project can run with limited funds. It is like a big risk. This project needs good funding as the prime work involves animals whose import and maintenance will cost a lot. When the budget table was overviewed, I guess the cost of printers and office work must be excluded as they are from the university funds. Even the laptops and hardware. The university or host institute has lots of PC which they can install in the monitoring room for data collection. In this case buy the Work bench software by Strawberry Tree along with strawberry Tree hardware for 2,500USD for the data collection that will cost approx. 9,000PLN that you were spending on laptops, computers, hardware’s etc. and reduce the cost. You can use a big separate room for such installation in the host institute rather than building enclosures for monitoring system that will delay your work by almost some 4-6 months. Plus, it will save money. More comments below*

It is a promising field of study. The old-world Rhesus monkeys are used since long time for several research due to large number of similarities with humans. Secondly, the authors point to the application of testing symmetry in a mammal that have 93% gene similarity with humans for the first time. Findings can be useful for evolutionary studies, even in studies involving animal behavior and learning.

It is a promising field of study. But the setup for the experiment like preparing an artificial enclosure with all necessary installments and all other stuff would definitely take 4-6months based on the work efficiency and supply given that the research has a time-span of 3 years. Even importing involves lot of paperwork, permission, and strenuous exercise of maintenance. -

The positive things about the project is that the authors wrote it nicely, justified what they want to achieve, even they created openings for PhD masters and technicians thus serving the society not only with knowledge but also employment. The good part is that they made a timetable just to keep everything on track.

Weak points of this project are the time-lapse in case of Task1 and Task2 can delay the time and may hamper their yearly planner. Secondly why the authors cannot switch to a different animal like rodents who are genetically, biologically, and behaviorally similar to humans. Even their procurement, ethical issues and maintenance is less strenuous than with the Rhesus Macaques. The summary was shorter with 131 words.

In the outcomes the authors said that the study will be fruitful in the future but also they could have highlighted some outcomes they believe could happen based on previous literature survey although it’s a new attempt but providing assumption is always welcomed like something they have explained in the hypothesis part.

In the significance part the authors have not justified the fields of science that will benefit from this research and whether this study has any clinical implications.

*In the timetable the rearing of rhesus monkey is for two years up to 15th March 2022. But the authors planned a 3rd experiment with newborn monkeys and their Mothers, aren’t they be included for rearing in the planner/timetable at least for the first half of the third year when they calculated the cost of maintenance as 4117200 PLN for three years.

What are the criteria on group division in the three experiments with the 1st experiment having 80 individuals? What level of conservation and security will be granted to these monkeys after the experimental period?

Finally, going by the breeding success of rhesus monkey i.e females produce one young each year how the authors will support the sample size of 20 1-year old monkeys per group? Also confusing is the mention of renting zoo enclosure for 1.5 years for maintenance of monkeys in an experiment that will run up to 2023. Why the maintenance is limited up to 1.5 years. Secondly the calculation is not clear because if rate per room is 5,000PLN for 10 rooms per month. So, for 1.5 years i.e 517 days it will be $5000 \times 517 = 2585000$ PLN and it can be sky high at the end of three years.

Why have you chosen business trips to the US provided their large number of research use with monkeys? Shouldn't it be fit for internships also?

Monika Opalek

1. Assessment of the scientific level of research or tasks to be performed

Research described in the proposal entitled "The mechanisms and origin of symmetry detection" prepared by Tomasz Gaczorek, Ewa Szlachcic and Sandra Åhlén Mulio, are important to fill existing knowledge gap in cognitive research field. The authors plan to conduct three experiments on rhesus macaques (*Macaca mulatta*). Scientific level of research described in the proposal is high. The hypotheses proposed are interesting and worth investigating. The authors describe correct scientific methodology.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

Proposed research have profound potential in broadening knowledge about mechanisms and origins of symmetry detection. Conclusions would significantly contribute to research about cognitive skills of animals and possibly can be extrapolated to humans. Nevertheless, in my view, the novelty and innovative character of proposed research had not been discussed in sufficient details. I suggest to include additional part to compare previously used methodology to the one proposed within this project

3. Assessment of the project feasibility

I assess that the probability of project feasibility is high. Time required for conduction of experiments is well planned. However, I would recommend to consider hiring additional technical support as well as a veterinary specialist, who are qualified and experienced in working with macaques.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Experiments conducted on large mammals, such as rhesus macaques, are very expensive. Clearly, such cost must be paid to ensure adequate animal maintenance, and proper facilities' qualities. Majority of costs listed within the budget would be spent on macaques maintenance. From that perspective I would argue that planned experiments could potentially include some additional factors, which would clearly increase scientific value of the project, simultaneously only slightly rising overall costs.

5. Strengths of the proposal

Overall, the project is well prepared. I would like to highlight following strengths of the proposal:

- Chosen topic is interesting and has profound potential for broadening knowledge in cognitive research field.
- Hypothesis are correctly formulated, and planned experiments enable their verification
- Experiments are thoughtfully planned, and described in details
- Scientific methodology is well described, following actual ethical recommendations and scientific methodology
- Tasks within each experiment are well defined and planned, however it would be helpful to also repeat tasks numbers in text describing research methodology
- Large sample size allow for strong statistical inference
- Budget is well planned
- Apart from scientific value, results of this studies can have potential application in improvement breeding programmes for animals in captivity (however, this aspect should be discussed more broadly in the proposal – details below)

6. Weaknesses of the proposal

Summary: I encourage authors to extend the summary with additional, more detailed information – especially:

- highlight and describe in more details hypothesis that would be tested within the project,
- shortly justify choosing rhesus macaques as experimental species,
- line 8: “non-human animals” is a very broad group, please be more specific, maybe give examples of animals that had or had not been tested so far,
- line 14-15: planned experiments do not test sexual attractiveness, so how would your research contribute to this field?

Other, general comments:

- Choosing rhesus macaques as experimental species had not been justified enough in the text. You mention that it had been shown that they detect symmetrical objects (lines 123-124), however there are also other species that can do so. Please, provide more details explaining your decision. Also current state of knowledge about cognitive skills of macaques, especially including symmetry detection abilities, should be discussed more broadly.
- As mentioned in line 84, not only symmetrical shape, but also colourful ornaments play important role in perception of symmetry in insects. Consider including symmetrical and asymmetrical colouring of shapes that macaques choose in experiments I and II as additional factor in your research. I believe that such modification would add another significant dimension and extra value to your research, simultaneously only slightly increasing workload and costs.

Lines 30-36: Please explain how your research relate to this possible explanations and whether you will test any of those possibility.

Line 76: Please explain how your research results can be extrapolated to conclude about human cognitive skills' evolution

Line 82: Experiments do not include testing sexual preferences, please explain how would you conclude about “how rhesus macaques and potentially other animals use or do not use symmetry when choosing a mating partner”?

Lines 85-86: Limited number of individuals suggest, that even “non-symmetrical” mates need to be included in such breeding programmes? Possibly, your research have implications for improvements in breeding programmes, however it is not explained in the text

Lines 88-89: As I understood, in your experiment III, you do not include “well-being” of macaques breed in symmetrical or asymmetrical environments – so the research do not provide information whether such arrangements can or cannot improve animals' conditions in breeding programmes

Line 99: It is not clear from the project description what is the aim of such internship

Lines 100-101: I believe you would “maintain” macaques for whole project duration, not only from 2nd to 15th moth of project? As I understood, during this period you will train macaques to recognize object with given number of symmetry axes? Please describe in more details task 2.

Lines 115-117: Specify whether extra time had been considered in case of failure of some training lectures from reasons not connected with experimental manipulations

Lines 124-125: Please, explain what is the nature of novelty in methodology or experiment design in your research, in comparison to cited publications or previous approaches to symmetry detection research

Final project proposal

The characteristics and origin of symmetry detection in rhesus macaques (*Macaca mulatta*)

Tomasz Gaczorek, Ewa Szlachcic and Sandra Åhlén Mulio

Summary

BACKGROUND The sense of symmetry is inherently related to humanity as it plays a central role in our aesthetic preferences. Previous research has provided evidence that non-human animals can also detect it. Its role is suggested to be intimately connected with sexual selection as symmetrical objects tend to be perceived as attractive. However, the actual pattern of recognition and its origin remains unknown.

RESEARCH QUESTIONS Symmetrical objects are abundant in nature. Most of them reflect vertical bilateral symmetry (VBS). In the first part of the proposed project we will investigate whether the prevalence of VBS affects the symmetry detection. Additionally, we want to examine whether the sense of symmetry is an innate evolutionary attribute or it is just an effect of the overwhelming surrounding.

METHODS We propose three experiments conducted on rhesus macaques (*Macaca mulatta*) which are known to efficiently perceive the symmetry. In the first experiment we will use figures with different orders of symmetry to check whether bilateral detection is the one preferable. In the second test, we will rotate the set of symmetrical and asymmetrical figures to scrutinize the expected advantage of vertical symmetry detection. The last experiment will include rearing macaques in the artificial environments abundant of symmetrical or asymmetrical objects to explore their subsequent preferences.

RELEVANCE The outcomes of the proposed study will hopefully contribute to a better understanding of the evolutionary processes resulting in contemporary aesthetic preferences. Also, it will potentially have a significant impact on studies related to sexual selections (mainly sexual attractiveness) and cognitive mechanisms.

1. Scientific goal of the project

Symmetry is common in nature and became the object of research concerning the phenomena of symmetry detection and speculation about its mechanisms (Thomas et al., 1992). There is growing evidence that organisms are sensitive to symmetries (Enquist & Johnstone, 1997). Many studies of symmetry preferences have focused on attractiveness. Symmetry in males' faces, body parts or ornaments is perceived as attractive by females (Møller, 1992; Møller & Thornhill, 1998). Female preferences for male symmetry have been found in humans (e.g. Jones et al., 2001; Scheib et al., 1999), zebra finches (Swaddle & Cuthill, 1994), and other species (Møller & Thornhill, 1998). However, there is also research showing preferences for symmetry and ability to detect it in non-mating contexts in different animals, for instance bees (Giurfa et al. 1996; Lehrer, 1999), spiders (White & Kemp, 2020), pigeons (Delius & Habers, 1978; Delius & Nowak, 1982) or dolphins (Thomas et al., 1992).

There are several alternative explanations for the occurrence of the similar symmetry preferences in nature. Firstly, preference evolved for adaptive reasons connected with mate choice (Johnstone, 1994) as the degree of symmetry provides information about the quality of the signaler (Little & Jones, 2006). Secondly, symmetry preferences can arise as a by-product of common properties of biological recognition systems (Enquist & Arak, 1994) such as e.g. generalization (Enquist & Johnstone, 1997). Thirdly, symmetry preferences can arise as a by-product of the need to recognize objects regardless of their position and orientation in the field of view (Enquist & Arak, 1994).

Bilateral symmetry is the most common symmetry observed in animals and is highly conserved during the evolution of animals (Li et al., 2020). It is easier to recognize it when the axis of symmetry is vertical in relation to the retina than the symmetry of the other orientations (Herbert & Humphrey, 1996). Bornstein et al. (1981) stated that the ability to recognize vertical symmetry in humans may be innate, mature very quickly or very easy to learn. However, the origin and mechanisms of the symmetry detection remain unclear.

Our research questions involve different characteristics of the symmetry sensing.

The first question concentrates on the detection mechanisms. **We investigate whether the pattern of detection depends on what is frequently met in nature (including own body shape).** We expect that macaques will more easily detect vertical bilateral symmetry (Fig. 1b) than any other. To test it we will verify two hypotheses:

I. Macaques are better in recognizing shapes with one symmetry axis than asymmetrical ones or shapes with a larger number of axes.

II. Macaques' ability to distinguish between symmetrical and asymmetrical objects decreases with increased deviation from the vertical position.

The other question is related with the emergence of symmetry detection. **We want to examine whether it is an effect of conscientious learning based on the surrounding environment or an innate evolutionary response.** The expected outcome of the experiment would not support an innate ability to detect symmetry if:

III. Both groups of macaques (raised in the environment fulfilled with symmetrical or asymmetrical objects) will show similar attachment to the environment of the initial type.

2. Justification and significance of the project

Animals perceive their world differently as they are affected by varying stimuli. Many vertebrates such as primates and humans rely heavily on visual cues to receive information about food, potential threats, conspecifics and when choosing a mate. Understanding the underlying evolutionary mechanisms of how such information is gathered is essential for our knowledge about their ecology and behaviour. Our project will be the first to investigate whether the ability to detect and preference for bilateral symmetry is an innate state of the rhesus macaques (*Macaca mulatta*). Furthermore, symmetry is an abstract visual pattern, i.e. because symmetry relies on the relation of objects together rather than the properties of the objects themselves (Thomas et al., 1992). Our project will identify not only the degree of taught perception of an abstract concept, but also an innate ability to perceive it regardless of training and thus, to some degree, understand abstract concepts. Understanding the nature and utilization of bilateral symmetry in rhesus macaques will provide valuable insights into the evolution of a visual aid and how it compares between old world monkeys and humans.

Our findings will:

- Provide new insights into human and animal evolution by understanding how new traits can be created as by-products of other necessary features of survival. As suggested by (Enquist & Arak, 1994), a preference for symmetry as a phenomenon can sometimes only be discovered if the objects of the preference appear in existence. Thus, detection and utilization of bilateral symmetry prompt science to further investigate such instances.
- Further provide an understanding of how rhesus macaques and potentially other animals use or do not use symmetry when choosing a mating partner. It has been shown in humans that bilateral symmetry is correlated to attractiveness and health, and as such, act as an important visual input when choosing a partner. In insects, symmetrical ornaments that are more symmetrical have been shown to be more attractive. Therefore, this could have further implications in helping wild animals in captivity with successful breeding such as in many conservation breeding programs in zoological gardens since individual specimens options are limited. As such, the limited breeding success may in part be due to potential mates presented not being symmetrical enough. If symmetry can be measured in individuals, there is a potential increase in successful breeding.
- Provide a foundation for future studies on other vertebrates and the potential improvement of aforementioned captivity breeding programmes for endangered species.

3. Concept and work plan

The general work plan of the project will include the following tasks:

Task 1: Internship in the institution conducting research on rhesus macaques e.g. Tulane National Primate Research Center in Covington, Louisiana, US. It will provide us experience in working with our model species rhesus macaques (*Macaca mulatta*).

Task 2: Preparation. This task includes preparation of facilities, enclosure, acquiring necessary supplies and training of co-workers.

Task 3: Maintaining rhesus macaques. After acquiring necessary supplies we will begin maintenance of rhesus macaques, which mainly requires feeding, cleaning and regular health check-ups by a veterinarian.

Task 4: Measuring the rhesus monkey’s ability to detect different orders of symmetry (Experiment I). This task will provide information for testing hypothesis I.

Task 5: Analysing the results from the first experiment. This step is indispensable for starting the second experiment.

Task 6: Investigating how the orientation of an object affects detection of its symmetry (Experiment II). This task will provide information for testing hypothesis II.

Task 7: Conducting the third experiment which is divided into two parts: rearing animals in three environments, fulfilled with symmetrical, asymmetrical or mixed objects for one year after which observations of time spent in the three environments by free choice will be recorded. This task will provide information for testing hypothesis III.

Task 8: Analysing results and preparing publications.

Projected time of the tasks is visualised in table 1.

Table 1. The timetable of the project.

Calendar date (month-year)	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23	May-23	Jun-23	Jul-23	Aug-23	Sep-23	Oct-23	Nov-23	Dec-23			
Tasks / Month in order	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36			
1. internship																																							
2. preaparation																																							
3. maintaining monkeys																																							
4. conducting experiment I																																							
5. analysing results																																							
6. conducting experiment II																																							
7. conducting experiment III																																							
8. analysing results																																							

Our project involves little risk as we will follow established guidelines and protocols of animal testing in regards of animal health and care to avoid injuries and disease of animals and staff as by the EU directive 2010/63/EU. Any facilities or tools used in experiments will be designed following the same guidelines. We will do a one-month internship to gain relevant experience. Additionally, we will employ a technical assistant with experience in handling primates in research as well as making sure to have regular health check-ups by a veterinarian.

4. Research methodology

All subsequent experiments will be performed on rhesus macaques (*Macaca mulatta*, Fig. 1a) raised and kept in captivity. The model organism was chosen based on previous research showing its abilities to detect symmetrical objects. However, the sensitivity of detection varied significantly between experimental designs and presented objects (Waite & Little, 2006; Sasaki et al., 2005). After experiments the macaques will be donated to other research projects that require rhesus macaques and that have all their permissions and paperwork in order since the demand for rhesus macaques is very high. Some individuals may be donated to zoological gardens if they are at capacity to house them.

All studies presented below are planned to be carried out on 20 1-year old macaques per each experimental group. The sex ratio within each group will be 1:1. In total: I (80 individuals), II (60 individuals) and III (60 individuals). The chosen number of individuals will provide the robustness to stochastic effects and a significant statistical power of applied tests. It would require standard equipment for studies made on non-human primates (as described in EU recommendations - 2007/526/WE).

I. The first experiment focuses on the detection of different orders of symmetry. Macaques will be exposed to the four, one-color buttons of distinctive shape, each having a variant number of symmetry axes (0,1,2 or 3) from which at least one is vertical. Depending on the experimental group (20 individuals), monkeys are going to be granted with food after pressing a button with desired symmetry order. Both button position and its shape will change each day (repetitions allowed) but the representation of different symmetry orders within a set will remain constant. After 2 weeks of learning (the timespan based on Bertrand, 1967), monkeys will be exposed to shapes of buttons never seen before but the food award will still be correlated

with a given number of symmetry axes. Then, the time until they reach food and the sequence of buttons pressed will be recorded. (Fig. 2a)

II. The second experiment will determine how the orientation of an object affects detection of its symmetry. Based on the first study, only one well-detected, symmetrical shape will be used further. Then, its two modified asymmetric counterparts will be created with an increased or decreased total figure area by approximately 5%. The modifying value will be chosen to be significantly higher than asymmetry commonly met in nature but simultaneously not changing the figure shape drastically. Macaques will be initially trained for 2 weeks to recognize a given symmetrical shape among other, slightly differentiated figures. The protocol will be similar to the one for the first experiment. However, to avoid an attachment to any figure position their orientation will change randomly during the learning stage. After training, monkeys will be exposed to 3 buttons of shapes corresponding to the initial symmetrical figure and two modified counterparts. Depending on the experimental group (20 individuals), shapes will be rotated by either 0, 45 or 90 degrees in relation to the initial vertical position. Then, the time until they reach food and the sequence of buttons pressed will be recorded. (Fig. 2b)

III. The last experiment will examine whether the nature of symmetry detection is exclusively related to conscientious learning based on the surrounding, highly symmetrical environment or it is an innate evolutionary response. To test it, 3 groups of macaques will be kept in small groups of 10 (with mothers) for 1 year after birth in the environments created of symmetrical, asymmetrical or mixed objects. If separated earlier from their mothers the young macaques would suffer psychologically. Also, the created environments will be big enough to overwhelm the individuals with the artificial objects. After this stage they will be given an opportunity to choose between a symmetrical or asymmetrical milieu. Importantly, the objects within the target environments will be different from those present in the initial one. The observations will be conducted by measuring the time spent in each target environment during one day. Note that all environmental characteristics (other than the object shape) will be constant throughout the experiment (Fig. 2c).

Observations gathered in each experiment will be analysed with the R software (R Core Team) with the use of standard statistical tests as t-test, chi-square test and analysis of variance (ANOVA).

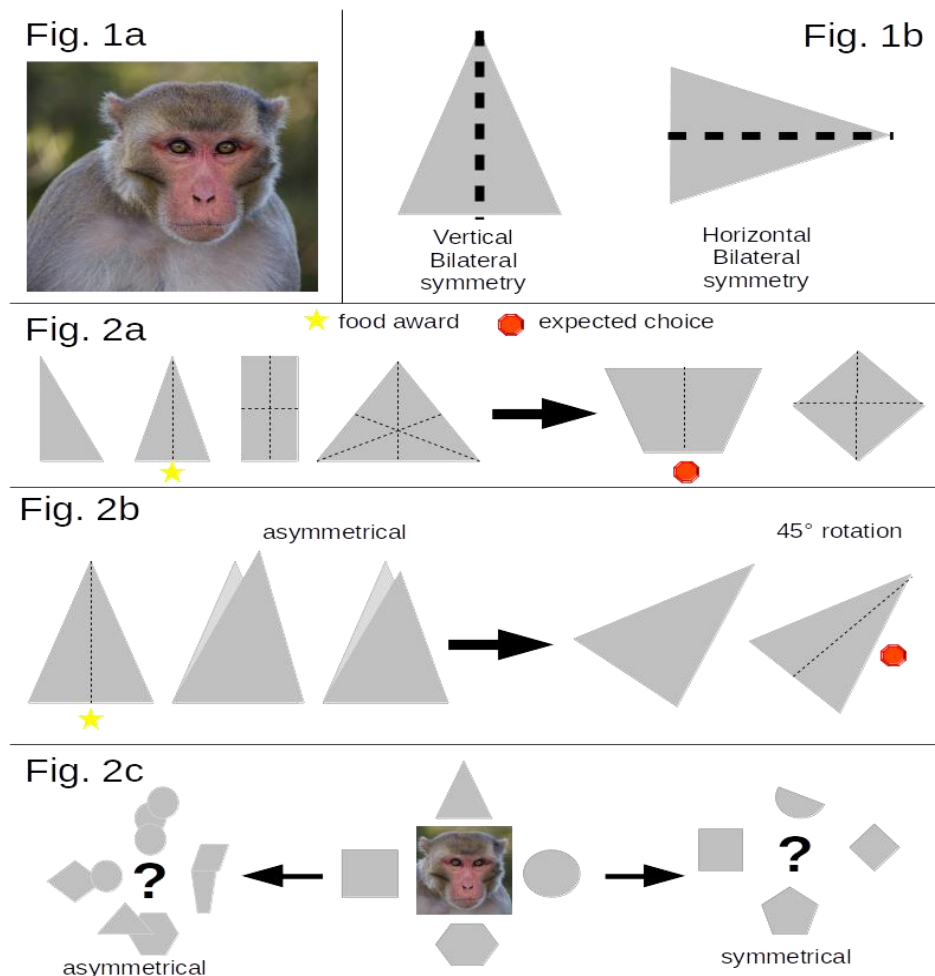


Figure 1-2c. Model organism and general scheme of experiments.

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Table with budget of the project

No.	Item	Funds for each budget year (PLN)			
		2021	2022	2023	Total
1	Direct costs, including:	8 686 245	2 235 000	1 932 000	12 853 245
	- Salaries and scholarships	264 000	264 000	240 000	768 000

	- Equipment and software	956 500	540 000	240 000	1 736 500
	- Other direct costs	7 465 745	1 431 000	1 452 000	10 348 745
2	Indirect costs (20%)	1 737 249	447 000	386 400	2 570 649
	Total costs (1+2)	9 543 128	3 091 883	2 788 883	15 423 894

Breakdown of project costs including justification and relevance for the tasks in the project Investigator/staff costs

Principal Investigators salaries: 540 000 PLN (3 PIs × 36 months × 5000 PLN)

Scope of work: planning, coordination, supervision of research, training and teaching master students, participation in gathering data, writing manuscripts

Project-related remuneration period: 36 months

Salary cost for one PI: 5000 PLN/month

Technical assistants salaries: 180 000 PLN (2 TAs × 36 months × 2500 PLN)

Scope of work: daily maintenance (e.g. feeding, cleaning), assistance during experiments, analysing videos, preparing facilities and enclosures

Project-related remuneration period: 36 months

Salary cost for one TA: 2500 PLN/month

Master students scholarships: 48 000 PLN (2 MSc × 12 months × 2000 PLN)

Scope of work: collecting of data in two experiments

Project-related remuneration period: 12 months

Scholarship for one MSc: 2000 PLN/month

2. Equipment/software

Renting zoo's facilities for the purpose of animals maintenance: 900 000 PLN (10 rooms × 5000 PLN/month × 1,5 year)

Creation of artificial enclosures including hall renting, maintenance and adjustment: 820 000 PLN (100 000 PLN initially + 20 000 PLN per month × 3 years)

Image recording set: 4 cameras, computer with software: 6000 PLN

Laptops: 9000 PLN (number: 3)

Hard drives: 1500 PLN (number: 3)

3. Other direct costs

Materials/Animals

Import and breeding monkeys: 13 800-38,640 PLN each, expected - 6 000 000 PLN in total

Cost of maintaining rhesus macaques per year: 1 372 000 PLN. Costs for three years: 4 117 200 PLN

Office supplies including printer cartridge: 1 000 PLN per year. Costs for three years: 3 000 PLN

Veterinary costs

Regular health check-ups and vaccinations: 1000 PLN per week, for 30 months 120 000 PLN

Unforeseen accident or illness: 30 000 PLN

Conferences and business trips

Costs of attending conferences of three PIs: 30 000 PLN

Internship in institution which conducts experiments on rhesus macaques: 34 745 PLN

(Flights (return tickets to US) 9 545 PLN, lodging 25 200 PLN for 1 month for 3 PIs)

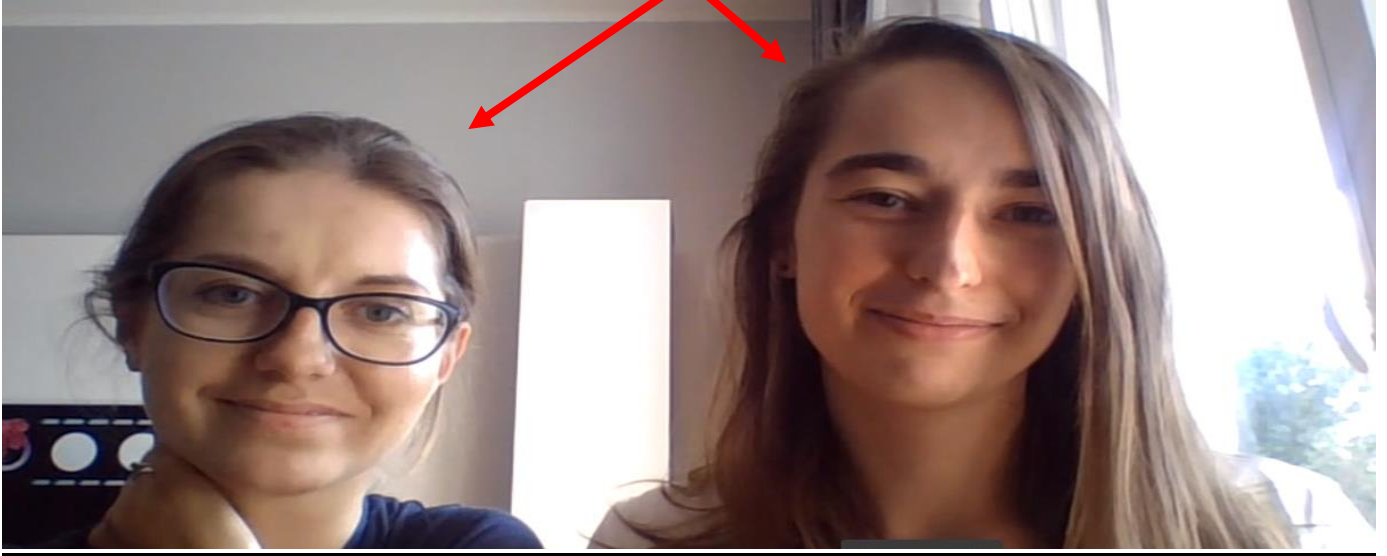
Other costs

Costs of publishing (including open access): 15 000 PLN

Project 4:

Is balcony vegetation significant for pollinators?

Monika Opalek & Monika Ostap-Chęć



Draft project proposal

Title: **Is balcony vegetation significant for pollinators?**

Applicants: **Monika Ostap-Chęć (PI), Monika Opalek (PI)**

Summary:

Insects abundance is rapidly decreasing worldwide. Governments and city authorities had undertaken various actions, such as sowing the flowery meadows or extensive mowing of lawns to improve pollinators situations in city areas. Plants grown on balconies and terraces are possibly additional source of pollen and nectar for insects. However, it had been not yet investigated whether pollinators indeed use them.

The project aims is to check whether **(1)** city areas with rich balcony vegetation increase pollinators abundance, **(2)** balcony plants are significant source of pollen for pollinating insects, **(3)** what is maximum balcony height to be visited by insects and **(4)** whether more alterative plants lure pollinators for higher flights than less attractive plants.

To test this hypothesis, we will arrange balconies with rich plant vegetation and monitor dynamic of insects' abundance and biodiversity in research zones representative for big cities. The insects will be collected by entomological nest and traps. Also, the plant species explored by *Apidae sp* (bees and bumblebees) will be identified by taking pollen sample form an insect and microscopic pollen examination.

Effective nature conservation is crucial nowadays. In highly urbanized city areas, there are limited possibilities to increase plant abundance. Rich balcony vegetation can be therefore potentially used as additional green areas in cities. The results of proposed research will provide information that can be used to arrange better our balconies and terraces, supporting nature conservation in the cities. Additionally, such social program involving citizens can be used for educational purposes to increase ecological awareness of the society.

1. Scientific goal of the project (description of the problem to be solved, research questions and hypotheses)

Description of the problem to be solved: Insects play a relevant role in all ecosystems' functioning. Their existence and abundance are essential for many processes, like pollination, herbivory, detritivory and nutrient cycling (Young & Gratton 2014). Insects are also important food source for higher trophic levels. One of the most important insects' function is pollination. About 80% of wild plants are estimated to depend on insects' pollination (Ollerton et al. 2011). What is more, insects cover even 30% food resources consumed by humans (McGregor 2002). Nowadays, due to habitat loss and fragmentation, as well as general deterioration of habitat quality and global climate changes, we observe rapid decline of insects' richness and diversity (Vanbergen & Initiative 2013; Hallman et al. 2017). Vulnerable for insect habitats are progressively lost also through progressive urbanization (Fattorini 2011; Matteson et al. 2012). Not only the areas of cities are expanding but also within the cities so far wild and vulnerable natural areas are paved over. In response, many city authorities try to undertake some actions to improve insects' conditions in large agglomerations. In more and more cities, roadside green belts are replaced with deliberately sown wildflower meadows. Parks and recreation places are arranged with vegetation supporting insects. Scientific studies confirm that such campaigns significantly increase abundance and biodiversity of wild pollinators (Daniels et al. 2020; Mody et al. 2020), but further solutions are still needed. Much is known about the terrestrial vegetation on the condition of pollinators, however nothing about the potential impact of balconies' and terraces' plants. Taking into account the high-density and mostly block

of flat housing in cities, balconies and terraces provide a relevant usable area. Green and full of flowers balconies can not only be a great decoration of buildings but also fulfill important ecological functions. Studies have shown that generally people are eager to arrange their balcony with plants, but rarely consider its utilities for pollinators (Krzymińska et al. 2020). Proper management of these spaces, providing plants that can be source of pollen and nectar, could significantly improve situation of insects in cities (Haaland et al. 2011; Samnegard et al. 2011). The results of research proposed here will provide crucial information about importance of good management of the balcony space, as well as can be used for environmental education and taking actions supporting nature conservation in the cities.

Within present project we aim to fill existing knowledge gap by **examining significance of balcony vegetation in the city areas for insects' abundance and biodiversity**. We will test following hypothesis:

Hypothesis 1 – City areas with rich balcony vegetation increases pollinators abundance and biodiversity.

Hypothesis 2 – Pollinators collect pollen from plants located at balconies (higher than 2m above ground)

Hypothesis 3 – Pollinators do not visit flowers located higher than 20 meters above the ground

Hypothesis 4 – More attractive plants lure pollinators for higher flights than less attractive plants

- 2. Significance of the project** (state of the art, justification for tackling a specific scientific problem, justification for the pioneering nature of the project, the impact of the project results on the development of the research field and scientific discipline);

State of art: Insects provide many ecological functions crucial for proper functioning of ecosystems. They are essential for processes such as pollination, detritivory, herbivory or nutrient cycling. Certainly, for people the most important function is pollination. About 80% of wild plants are estimated to depend on insects pollination (Ollerton et al. 2011). What is more, insects cover even 30% food resources consumed by humans (McGregor 2002). The main group of pollinators are wild bees and bumblebees (Apoidea), butterflies (Lepidoptera), some flies (Syrphidae) and beetles (Coleoptera) (Rader et al. 2016). It is well documented that abundance and biodiversity of pollinators progressively decrease. In the cities, authorities undertake variety of actions trying to protect them. Replacing roadside green belts with deliberately sown wildflower meadows brings positive effects (Mody et al. 2020). Arranging parks and recreation places more insects' friendly or extensive lawn mowing also play an important role (Daniels et al. 2020). Even small areas, covered with plants attractive for pollinators can have profound impact on insects. Therefore, balconies could be considered as additional areas that will support pollinators, especially since many plants used to sow in flowery meadow can also decorate the balconies.

Scientific problem: The main research question of this project is whether balconies with rich vegetation can increase abundance and biodiversity of pollinators. For this purpose, we want to analyze if enriching the given area with the additional flower area on the balconies will increase the abundance of insects. At the same time, we want to investigate if pollen collected from flowers located at balconies, in comparison to other food sources located at low altitudes, play a significant role in the insects' diet. Second part of research will examine maximum height that pollinators can fly in order to collect nectar or pollen and whether more attractive "food supplier" lure insects to higher flights.

Justification for tracking proposed scientific problem: Efficient nature conservation is crucial nowadays. Rapidly decreasing abundance and biodiversity of insects is one of the major problems especially in highly transferred areas, such as big cities. Governments and city authorities had undertaken multiple actions to preserve vulnerable green areas. However, in highly urbanized zones, there is little possibility to increase plants abundance. Properly arranged, rich balcony vegetation can become significant nutrient source for insects. Still, it has not been checked yet whether insects indeed explore plant nutrient sources located at higher altitudes. Within proposed project we want to monitor dynamic of insects' abundance and

biodiversity after significant increase of balcony vegetation in representative research zones. What is more, we will determine maximum height of plants location, which are still explored by insects and whether this height is influenced by plants' attractiveness. Proposed research will fill existing knowledge gap in ecology and entomology field as well as can have applicational relevance.

Pioneering nature of the project: It has been shown already that even small greenbelts with flowering plants increase insects' abundance and biodiversity (Mody et al. 2020). As such, plants located at balconies are another potentially significant nutrient source for many insects. However, it has not been tested yet whether insects, especially pollinators explore pollen and nectar sources located at higher altitude. In this project we want to determine whether increased balcony vegetation in city areas with high and low urbanization would increase abundance and biodiversity of insects. What is more, we want to fill existing knowledge gap and check what is the maximum height, that insects reach to collect pollen and whether plant attractiveness influence this maximum height.

Scientific importance for development of research fields: Results of the first part of the research (testing hypothesis 1&2) will provide important information that can be used in subsequent studies about urban areas' management. The next step after project completion, our research questions should be extended to further experiments of other urban spaces (such as roofs area or special terraces with attractive plants) for potential use for pollinators support. Results of second part of experiments (testing hypothesis 3&4) will make a significant contribution to the state of knowledge about food-gaining ability of pollinating insects. Knowledge of the insects' maximum flight heights and their potential ability to distinguish between profitable and less or no profitable flowers above the ground can be used in further studies both in ecology and evolution fields (for example in research testing life strategies of various insects).

Social relevance: Apart from scientific significance, presented research have applicational relevance. Management of green areas in cities is a challenging issue. Some actions, like sowing the flowery meadows or extensive mowing of lawns had already been proved to increase insects' abundance and biodiversity. Conclusions made based on our proposed research may be next important source of information for governments and city authorities. Citizens eagerly grow plants at their balconies. If the most efficient way of balconies arrangements can be identified, it can be become both important action for nature conservation in cities and society educational program increasing ecological awareness.

3. Concept and work plan (general work plan, specific research goals, results of preliminary research, risk analysis);

General work plan:

Part 1 - Testing hypothesis 1 & 2: The research zones would be located in all polish cities with number of citizens higher than 250 000 (11 cities: Warszawa, Kraków, Łódź, Wrocław, Poznań, Gdańsk, Szczecin, Bydgoszcz, Lublin, Białystok, Katowice).

Task 1 - Based on GIS data, we will choose 4 **research zones** of 1 km² (square area, 1 km x 1 km) in each city - two zones with high urbanization and two zones with low urbanization. We define high urbanization as an area where more than 50% of the surface is transferred, while low urbanization as an area where less than 25% of the surface is transferred.

Task 2 – During 1st year of the project (summer 2021) we will determine insects' abundance and biodiversity in all research zones, using traps. Additionally, we will collect *Apidae sp.* (bees and bumblebees) by entomological nets to examine which plants species are the most attractive as a pollen source in given research zone. We will also document all plant species already present in each research zone.

Task 3 – Within chosen research zones, in 2021, we would recruit volunteers, who are willing to

participate in the project (during whole project duration from 2021-2023) and agree for balcony arrangement. The chosen balconies cannot be located higher than at 3rd floor.

Task 4 - In spring 2022 we will arrange chosen balconies in all research zones. Within each 1 km² research zone we want to introduce additional plant surfaces of ~400 m², having ~1 m² of plant surface at each balcony (1 m² is an equivalent of 4 oblong flowerpots size 1 m x 0.25 m). We plan to use following plant species: *Lavendula sp.*, *Centaurea cyjanus*, *Thymulus serpyllum*, *Nepeta cataria*, *Echinacea purpurea*, *Ranunculs sp.* *Leucanthemum vulgare*, *Knautia arvensis* to arrange balconies.

Task 5 – To verify **hypothesis 1** we will monitor dynamic of insects' abundance and biodiversity in areas where we significantly increased plant vegetation at balconies. We will use traps set up 3 times during each summer season.

Task 6 – Using entomological nets, we will collect *Apidae sp.* 3 times during each summer season, to check whether insects explore pollen source arranged at the balconies (**hypothesis 2**). Plant species will be identified by microscopic examination of pollen.

Part 2 - Testing hypothesis 3 & 4: The experiment to verify hypothesis 3 and 4 would be performed in 2023.

Arranged balconies would be located between 1st and 10th floor of block of flats.

Task 7 - We will choose 4 estates to set up experimental arrangement of balconies. The estates would be located in Kraków, and have moderate level of urbanization (~30% of transferred area). Within each estate, we want to arrange all balconies in four blocks of flats. Chosen four blocks of flats need to have the same building plan and at least 10 floors high. Each block of flats would have balconies arranged differently.

Arrangement 1 - All balconies in one vertical segment are arranged with ~2 m² of **highly attractive plants** (*Lavendula sp.*, *Centaurea cyjanus*, *Thymulus serpyllum*, *Nepeta cataria*, *Echinacea purpurea*, *Ranunculs sp.* *Leucanthemum vulgare*, *Knautia arvensis*).

Arrangement 2 - All balconies in one vertical segment are arranged with ~2 m² of **less attractive plants** (*Begonia serpensflores*, *Petunia sp.*, *Tagetes panula*, *Tagetes tenuifolia*, *Tagetes erecta*, *Fuchsia sp.*, *Dahlia sp.*, *Lobelia erinus*, *Lobularia maritima*).

Arrangement 3 – Only balconies at given floor (height) in all segments are arranged with ~2 m² of highly attractive plants (the same species composition as in arrangement 1).

Arrangement 4 – Only balconies at given floor (height) in all segments are arranged with ~2 m² of less attractive plants (the same species composition as in arrangement 2).

The arrangements 1 and 2 will be fixed during whole spring and summer season. The floors for arrangements 3 and 4 will be changed weekly in random order.

Task 8 – Data collection – For arrangements 1 and 2 we will record 3 weeks - one week per month in June, July and August, but analyze only one day with optimal weather conditions (sunny, warm day, without rain). For arrangements 3 and 4 we will record whole week with plant arranged at give height but analyze only one day with optimal weather conditions (sunny, warm day, without rain). All video records will be taken during daytime.

Research goals:

Hypothesis 1: We aim to describe dynamic of species abundance and biodiversity in research zones during project duration – before balcony arrangement (summer 2021), in first year with arranged balconies (summer 2022), in second year with arranged balconies (summer 2023).

Hypothesis 2: We want to learn whether plants located at higher altitudes (more than 2 meters) can be a significant pollen source for bees and bumblebees

Hypothesis 3: We will establish maximum altitude of plant location at which plant are visited.

Hypothesis 4: We want to determine whether pollinators are more eager to fly higher to reach more attractive plants.

Risk analysis: We estimate overall risk of the project as low to moderate. Involvement of the volunteers that agreed for balconies arrangement introduces a possibility that some plants would be grown incorrectly. We will provide information leaflets for volunteers to reduce risk of plant death. Additionally, we included in the budget costs for replacement of 60% of plants in part 1 for second experimental season (summer 2023). As the project involve field observations, weather conditions may also force us for changes in the schedule. Extra time had been booked for such possibility. Other parts of the project are at very low risk. Insect and pollen species collection and identification are standardized methods, which are also frequently used by our research team. We also have experience in video data analysis.

4. Research methodology (underlying scientific methodology, methods, techniques and research tools, methods of results analysis, equipment and devices to be used in research);

Research zones: For testing hypothesis 1 and 2 we will choose 44 research zones (11 polish cities, 4 research zones in each), sized 1 km x 1 km. Half of the zones would be highly urbanized, and latter half would be with low urbanization. The zones would be chosen based on publicly available GIS data of the cities, where the surface is divided into green and transferred areas. We define high urbanization as an area where more than 50% of the surface is transferred, while low urbanization as an area where less than 25% of the surface is transferred. For part 2 of the project (testing hypothesis 3 & 4) we will use GIS data to determine surface urbanization of chosen estates.

Data

collection:

Entomological nets – Using entomological nets is a common method to collect flying insects. We will use them to complete tasks 2 and 6. Caught insects would be immediately placed into separate tubes, so that pollen samples could be analyzed later under a microscope. Both insects and plant species would be identified.

Traps – We will locate traps on the ground level, and they will be used to complete tasks 2 and 5. Caught insects' species would be identified later in the laboratory.

Cameras – Videos will be used to complete task 8. We will identify insects' orders which appeared on recordings.

Data analysis: For testing hypothesis 1 and 2, we will statistically analyze dynamic of insects' species abundance and biodiversity before experimental treatment (balconies arrangement), during first and the following year after balconies arrangements. For testing hypothesis 3 & 4 we will determine significant differences between abundance of insects at chosen heights of balconies.

5. Project literature (a reference list for publications included in the project description, with full bibliographic data).

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- (4) Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE*, 12(10):

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- (6) Matteson KC, Grace JB, and Minor ES. (2013) Direct and indirect effects of land use on floral resources and flower-visiting insects across an urban landscape. *Oikos*, 122: 682-694.
- (7) McGregor S E. (1976) Insect pollination of cultivated crop plants. United States Department of Agriculture, Washington DC.
- (8) Mody K, Lerch D, Müller A-K, Simons NK, Blüthgen N, Harnisch M (2020) Flower power in the city: Replacing roadside shrubs by wildflower meadows increases insect numbers and reduces maintenance costs. *PLoS ONE*, 15(6): e0234327.
- (9) Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? *Oikos*. 2011;120(3):321–326.
- (10) Rader R, Bartomeus I, Garibaldi LA, et al. (2016) Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 146-151.
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- (12) Vanbergen AJ, and Initiative IP. (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, 11: 251-259
- (13) Yang LH, Gratton C. Insects as drivers of ecosystem processes. *Current Opinion in Insect Science*. 2014;2:26–32.

Table with budget of the project

PROPOSED BUDGET

No.	Item	Funds for each budget year (PLN)			
		2021	2022	2023	Total
1.	Direct costs, including:	199 000 PLN	993 000 PLN	682 920 PLN	1 874 920 PLN
	- Salaries and scholarships	60 000 PLN	60 000 PLN	60 000 PLN	180 000 PLN
	- Equipment and software	30 000 PLN	60 000 PLN	-	90 000 PLN
	- Other direct costs	109 000 PLN	873 000 PLN	622 920 PLN	1 604 920 PLN
2.	Indirect costs (20% of direct costs)	39 800 PLN	198 600 PLN	136 584 PLN	374 984 PLN
	Total costs (1+2)	238 800 PLN	1 191 600 PLN	819 504 PLN	2 249 904 PLN

Breakdown of project costs including justification and relevance for the tasks in the project

Salaries and scholarships:

1. Salary for two PI's: 3 years x 12 months x 1 500 PLN x 2 PIs = 108 000 PLN
Scope of work: Experiment planning and tasks coordination, people management, supervision and training data analysis and interpretation, preparation of manuscripts,
2. Scholarships for two Master or Bachelor Students: 3 years x 12 months x 1 000 PLN = 72 000 PLN
Scope of work: field work, sample collection and laboratory analysis

Equipment and software

1. Laboratory and field equipment (such as entomological nets and traps, laboratory plastic): 20 000 PLN
2. Cameras: 30 cameras x 2 000 PLN = 60 000 PLN
3. Laptops: 2 laptops x 5 000 PLN = 10 000 PLN

Other direct costs

1. Balconies arrangements: Total: 1 147 920 PLN
 - For testing hypothesis 1&2: 11 cities x 4 research zones x 400 m² x 4 flowerpots (per 1m²) x 10 PLN (cost of one flowerpot) + 60% for plant replacement in next year = 1 126 400 PLN
 - For testing hypothesis 3&4: 4 estates x (10 balconies (arrangement 1) + 10 balconies (arrangement 2) + 5 balconies (arrangement 3) + 5 balconies (arrangement 3)) x 8 flowerpots (per balcony) x 10 PLN + 20% for plant replacement = 11 520 PLN
 - Planning and execution of balconies arrangements by external company: 100 000 PLN
2. Preparation of educational leaflets for volunteers who would agree for balcony arrangement - including graphical services, printing and distribution – 10 000 PLN
3. Scientific conferences – including travel costs, accommodation and conference fees – 5 000 PLN per conference x 2 conferences x 4 people (2 PIs and 2 Students) = 40 000 PLN
4. Publication costs – including linguistic proofreading and open access fee – 10 000 PLN
5. Other outsourcing (covering costs such as poster prints, graphical services, linguistic correction) - 10 000 PLN
6. Thirty-three people for field support in high research seasons – 3 months employment x 3 years x 33 people x 1 000 PLN = 297 000 PLN
Scope of work: recruiting of volunteers, collecting data from cameras/traps, supervision of balconies during summer season.

Reviews

Prof. dr hab. Jan Kozłowski

The title is, in my opinion, adequate and interesting.

Summary: Second and third paragraphs should be joined together. A paragraph in English is an important and not arbitrary entity, closed in a sense. Thus if the third paragraph starts with “To test this hypothesis ...”, it is not clear what the hypotheses (should be plural according to the previous paragraph) the authors mean writing “this”. But in general the summary is clear and fully informative. I would like to have the third paragraph a bit more developed.

The project: It is not my job to concentrate on details; I was asked for general overview of all four projects. I like the general idea of the project. The project is a bit messy in language and construction, but I understand the pressure of time. Why so many cities have been chosen, which would increase costs and could make the project unfeasible? Why not more samples within one city? Are cities considered thus repetitions or geographical position of them is an important but not specified factor? The budget seems too low for so many cities: traveling costs are not included.

Prof. dr hab. Wiesław Babik

1. Assessment of the scientific level of research or tasks to be performed

The project aims at using simple tools to test several interesting hypotheses centred on the pressing problem of remedying the pollinator crisis. The study is overall well designed and likely to provide appropriate tests of the research hypotheses.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

It's been surprising to learn that the effect of balcony vegetation on pollinating insects has not been studied – the project sets out to fill an important knowledge gap. The project is interesting and timely, likely to attract considerable attention and provide information of immediate use for managing urban biodiversity. While the hypotheses 1 and 2 are well thought off and likely to advance the field, I'm not sure whether hypotheses 3 and 4 as formulated are likely to improve our understanding of the problem. To assess the likely effect of balcony's elevation on pollinators, we would like to know the frequency distribution of visits, not just the maximum height.

3. Assessment of the project feasibility

The project is feasible, but I feel that the authors underestimated the risks. In particular, the project involves the need of extensive collaboration with the general public – inhabitants of the apartments, which is likely to be more complicated than the authors may think. The project appears extremely laborious. In this context, it is not clear to me what are the advantages of using so many cities with just four research areas in each – this is an important decision that should be well justified. Do the authors expect a large variation between the cities?

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

The cost estimates are precise and well explained. The project, however, is rather expensive. I'm not sure whether the costs are justified, because no good reasons for performing the study in as many as 11 cities were provided.

5. Strengths of the proposal

- Interesting, timely and under-studied topic
- Good overall study design
- Clearly formulated, testable hypotheses
- Large scale of the study (but see below)

6. Weaknesses of the proposal

- Too few details about statistical analysis in the context of study design
- Many repetitions, the same info appears in several sections. This is justified, but only to some extent – there are clear length restrictions, and some sections, such as state of the art are only briefly sketched
- The scale of the project possibly too large, making it exceedingly costly
- Risk assessment too optimistic
- English could be improved

Kyle Coughlan

Overall, a very nice proposal which requires some work. It is a very interesting topic and an ambition yet simple plan to further our knowledge of conservation of pollinators which is a very important topic in today's world and one which would gather significant attention and has the potential to alter how we approach the problem of insect decline.

1. Assessment of the scientific level of research or tasks to be performed

The proposed project is of a high scientific level and if carried out as planned with the desired data collected and analyzed correctly would in no doubt be published by a leading journal in any number of field including entomology, conservation biology and animal behavior. Research such as this even has potential to reach fields outside of the biological sciences such as architecture and urban planning.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

Understanding insect, particularly pollinator ecology is very important, and I believe this study would help advance our understanding of this field. Gaining knowledge of foraging behavior regards to height, preference of pollen sources etc would be very beneficial to many governmental and public organizations globally. Helping to better plan and develop strategies for the conservation of pollinators

3. Assessment of the project feasibility

I believe that the authors have all the necessary knowledge and skills to perform all of the tasks listing within this proposal. While I think the project is feasible there is little information about how it would be achieved. For instance, targeting 11 cities in Poland seems like a lot of work. For task 3 it is not stated how recruitment of volunteers will be undertaken, or indeed how many they wish to use. While I have no doubt people would be willing to allow the researchers to build a nice flower arrangement on their balcony I am not sure they would be willing to have people coming into their homes to monitor the insects or even how that would be planned out, and have doubts that they will look after the arrangements properly. Also, a lot of people rent their apartments and it may be hard to find enough volunteers in each city who live in these areas.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

I am not entirely convinced the costs to be incurred are wholly justified. The use of 11 different cities seems excessive and no explanation is put forward as to why the authors wish to carry out such an extensive study considering the plan for each city is the same and merely represents replication rather than answering different questions. Perhaps more can be learnt by reducing the replicants and gathering more detailed data on a smaller

number of locations. This would surely greatly reduce the cost and make it easier to plan and manage while also increasing the scope of the study further.

5. Strengths of the proposal

- The authors have identified an issue, namely insect decline and have not only found a gap in the knowledge regarding research in this field but one which also provides a way of contributing and fixing this issue. It is an ambitious project but one which could hold many benefits and would undoubtedly produce workable and useable data and further develop the field.
- It is a very well thought out and detailed proposal to study a problem which is of great importance in today's modern world. Both the decline of insects and our ever-greying city landscapes.
- It taps into an increasing trend of planning with pollinators in mind which will capture public attention and can educate the lay community on how best to help.

6. Weaknesses of the proposal

- As a native English speaker, it is natural to notice little grammatical, spelling and syntax errors in the proposal. Small things like a, an, and the are missing in quite a few places. As well as this there are several errors in general, for instance in the first sentence of the summary it is stated that extensive mowing of lawns improves pollinator actions which surely is an error in wording, another is McGregor 2002 mentioned in the first description but in literature it is from 1976? Taking into account that this was written under such a strict deadline and is just the first draft it is not worth fussing over right now but I would suggest you have a native speaker and/or an extra associate to run through it for corrections before handing in the final version.
- There is a lot of repetition in the proposal, for instance the description of the problem to be solved and the state of the art section contain paragraphs which are almost entirely the same content. I am not sure the state of the art section is for this content either. While I know this is a unique study, the topic of increasing pollinator diversity in cities is a very hot subject and there are a lot of projects ongoing regarding this field which can be used to fill out this section. Two that I know of which the authors may find interesting are a project by Trinity College in Dublin who replaced their inner city grass lawns with wild flower meadows earlier this year, and also perhaps look for information about the Bosco Verticale in Milan, a skyscraper complex covered in trees, I know there was one study done with birds on these buildings (Belcher et al. 2018) so might be worth a look.
- The data analysis section is very scant in information and it is not clear from the text how the authors wish to score the abundances or "attractiveness" of plants, they are listed as highly or less attractive but it is not stated if there is an order or scoring system, or whether this is a binary variable, or indeed if they will be scoring pollinator taxa. Also, they may have missed some ideas regarding data collection such as directional exposure, prevailing winds, and temperature logging which I am sure will also play a big role in the foraging behavior of insects and is data which will help remove noise from their models.
- I know there was a page limit regarding this proposal but I feel like a graph of the work plan may have helped, as the expression goes a picture paints 1000 words and it might have been nice to include one. For such a flowery topic, pardon the pun, it was short of colour. I think with careful editing of the text and the inclusion of an informative graph this proposal could be improved.

Paulina Joško

1. Assessment of the scientific level of research or tasks to be performed

Topic of the project is not only interesting, but also addresses an important issue of species conservation. Problem which this project concerns is well described by the authors. Scientific background and current state of the art are presented in a clear, logical way. All information included in the description is relevant to the topic of the project. The aim of the study is explicit and comprehensible, and the hypotheses are well-formulated. Work plan is carefully designed, with reasonable order of tasks. Methodology is suitable

to verify stated hypotheses. However, there are some aspects that require further explanation or improvement.

In the description to “task 1” and “research zones” authors specify the area of a single zone, but this choice is not justified by reference to proper literature or authors reasoning. What is more, in these parts additional factors such as minimal distance between research zones in one city or availability of habitats for pollinators in the area surrounding each research zone should be included, as they have an influence on data analysis and may influence the interpretation of the results. In “task 4” authors list plants they plan to use, but there’s no comment on why they chose those particular species. It is not defined how specifically pollinators would be identified – to the level of species, genus, family, etc.?

Furthermore, it would be worth mentioning whether authors are going to treat the abundance of all pollinators from one research zone as a whole while comparing the zones, or perhaps analyze the abundance of each pollinator group separately, because this aspect remains unclear to me. What I also don’t understand is why authors decided on a maximum height of 20 meters in hypothesis 3. This is an arbitrary choice, while the goal referring to this part of a project is to establish the maximum height, so why this particular height was set as an upper limitation? For the data analysis part, authors should indicate which statistical tests they intend to use to verify hypotheses.

Finally, a minor remark, I suggest that authors consider rephrasing the headings in “research goals” from “Hypothesis 1: (...)” into “Goal 1: (...)”, with added note that numbers of goals correspond to the same numbers of hypotheses, as current wording seems to be a little confusing.

2. Assessment of the project’s innovative potential and impact on the advancement of the scientific field/discipline

The knowledge gap is well defined and aims of the project are properly fitted in order to complete current state of the art. Innovative potential of the project is emphasized by the authors and supported by appropriate argumentation. In my opinion project bears a high scientific value and could contribute to advancement of environmental sciences, as well as have a positive impact on the education of the society or even economy.

Nevertheless, for the project to have an impact on the scientific field, the results need to be published. Authors include publication costs in their budget, but it is essential to declare that results obtained in the project are going to be published in the short description of the project. Authors should add that information to the text, preferably with details on the number of articles they intend to publish.

3. Assessment of the project feasibility

Duration of the entire project, scheme of subsequent tasks and division of tasks for each year are reasonable and, in my opinion, feasible.

One major issue which I’m concerned about is that for the project to be carried out a great number of volunteers who will be willing to participate and give access to their balconies is needed. Authors should elaborate more extensively on that issue in their “risk assessment” section. Perhaps authors will consider carrying out some pilot questionnaire among the citizens before they definitely decide on the locations of the research zones, in order to make sure, that sufficient number of people are interested in volunteering.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Overall, the budget is exhaustively justified, each section is detailed, and I don’t see any important cost to be missing.

The only confusing aspect to me is the breakdown of the scholarships for the students. It is specified that two students would be involved in the project and the calculations presented in line 258 show that both of the students would be remunerated for the entire 3 years of project duration. However, the timespan for completing the Bachelor dissertation is only one year and for Master thesis two years. Therefore, the costs here require either recalculation or further clarification.

5. Strengths of the proposal

- Problem is extensively described, hypotheses are properly defined and possible to verify, innovativeness and significance of the project are distinct and inherently justified
- Tasks are presented in logical, organized manner and are adequate to test the hypotheses
- Thoroughly and carefully written budget

6. Weaknesses of the proposal

- The risk connected with recruiting volunteers who are going to give access to their balconies is not discussed by the authors, when it plays a crucial role in the success of a project
- Some additional aspects (mentioned above, points 1-4) need to be taken into account by the authors or explained in greater detail
- Some minor errors need to be corrected before submitting the final version of the project, to give the authors a clue, I list couple examples below:
 - Recalculate the total amount in line 266
 - There is no need to place „PLN” in each cell of a table, when you define the currency in a heading, removing „PLN” from other cells would simplify the table and make it more clear
 - There are some inconsistencies in the reference formatting, such as: year of publication placed in a different order in line 247 than in other literature positions; in lines: 223, 230 and 244 you used “and” before the name of the last author, while in other positions it is not written, etc.
 - Double-check the spelling and grammar; line 15: “this hypothesis” – you refer to all 4 hypotheses, so change to plural “these hypotheses”; line 37: „Hallman”, while in line 225: „Hallmann”; lines 38-39: „Matteson et al. **2012**”, while line 230: “Matteson KC, Grace JB, and Minor ES. (**2013**)”, etc.

Ewa Szlachcic

1. Assessment of the scientific level of research or tasks to be performed

Proposed project touches on a very interesting and timely topic concerning the insects' conditions within large urban areas. Project is clearly written and structured. The experiments are well described and considered. There are links between each hypothesis and tasks which make the structure more clear and understandable. Project is properly justified, the knowledge gap is clearly indicated. State of art is well described.

Nevertheless, here are some of my comments about the hypotheses and tasks. For me, part of the first hypothesis “City areas with rich balcony vegetation” suggests, that you attempt to compare city area with rich balcony vegetation to an area with poor balcony vegetation and in that way it is not testable by experimental design. Perhaps it can be modified to state: “Increased balcony vegetation affects pollinators abundance and biodiversity”. Then my suggestions would be to include in the project proposal predictions about this hypothesis. Moreover, it is not justified in the proposal why in your research the focus is on two types of urban area (depending on the percentage of transferred surface). To the same point, no hypothesis corresponding to that fact can be found.

Regarding the second hypothesis I am not certain if it is testable in its current form. Perhaps, it should be put as in the summary: “balcony plants are significant source of pollen for pollinating insects” instead of the version placed in the Scientific goal of the project (“Pollinators collect pollen from plants located at balconies (higher than 2m above ground)”). In what way can it be investigated whether “pollen collected from flowers located at balconies, in comparison to other food sources located at low altitudes, play a significant role in the insects' diet”. It may be the case that the same plant species are present at low altitudes that those present at balconies. Would it not affect results of conducted experiments? As was claimed in the proposal, “many plants used to sow in flowery meadow can also decorate the balconies”, from which I assume that in the arrangements the same species as observed in flowery meadow will be used.

Another point that is not clear to me is why in the experiment to verify hypothesis 3 and 4 the floors of arrangements 3 and 4 need to be changed weekly. It also brings the question of how it will be performed (will the pots with plants be transferred every week between balconies?). Other than that the experiments are designed properly, in a way that they are doable.

One other possible doubt which I see is that the vegetation surface on others not arranged balconies can randomly vary between the years. Will this not affect the results? Or can it be considered negligible with the increase in vegetation surface by approximately 400 m²?

As a more general comment I wanted to pose this question: is the project's focus only on pollinators or does it spread to examining the biodiversity and abundance of all insects? There is a bit of discrepancy between hypotheses and objective of the project and planned tasks, as the hypotheses only concern the pollinators and there are no predictions about other species.

2. Assessment of the project's innovative potential and impact on the advancement of the scientific field/discipline

This project has a novel character and may have scientific relevance in its field. Results from this research can provide information about the ability of pollinators to explore plant nutrient sources located at higher altitudes and thus about the importance of managing such areas as balconies. Proposed project is applicable in its characteristics. It might be useful in helping with nature conservation in cities by proper management of urban areas and raising ecological awareness. Additionally, in their paper authors highlighted other possible future fields of study.

3. Assessment of the project feasibility

The project is feasible. The investigators will base their research on the standardized methods about insect and pollen species collection and identification. Moreover, they have already worked in this field of study and also have experience in analyzing the video data, which will be necessary for providing information to testing hypotheses 3 and 4. Risk analysis in the project has been done. Authors pointed out the possible problems and proposed the solutions how to solve them. However, I see one additional point that may affect feasibility. The possible risk is that during the last experiment not all of the owners of flats will agree to participate in research, and share their balconies or they can be simply not present at home during summer. It would require performing task 7 one more time and therefore that would mean wasting time.

4. Are the costs to be incurred well justified with regard to the subject and scope of the research?

Costs are presented in detail and have references to the planned tasks. However, it is not mentioned in the project on what basis the percentage of replacement plants was assumed.

5. Strengths of the proposal

- the topic is interesting and timely
- proposed project is well structured
- project has applicable character - results can help in nature conservation and planning of urban areas
- novelty of the idea
- quite detailed budget
- well justified significance of the project

6. Weaknesses of the proposal

- two types of urban areas do not correspond to any hypothesis
- the species *Centaurea cyjanus* is a one-year plant
- spelling mistakes and typos: importatnt (line 33), much in know (line 46), increasement (line 93), can be become (line 119), at give height (line 172)

Final project proposal

Title: **Is balcony vegetation significant for pollinators?**

Applicants: **Monika Ostap-Chęć (PI), Monika Opalek (PI)**

Summary:

Insects abundance is rapidly decreasing worldwide. Governments and city authorities had undertaken various actions, such as sowing the flowery meadows or extensive mowing of lawns to improve pollinators' condition in city areas. Plants grown on balconies and terraces are possibly additional source of pollen and nectar for insects. However, it had not been investigated yet whether pollinators indeed use them.

The project aims is to check **(1)** whether city areas with enriched balcony vegetation would increase pollinators abundance, **(2)** if balcony plants are significant source of pollen for pollinating insects, **(3)** what is maximum balcony height to be visited by insects and **(4)** whether more attractive plants lure pollinators for higher flights than less attractive ones. In the first part of our research we will arrange balconies with rich plant vegetation and monitor the dynamics of insect abundance and biodiversity in research zones representative for big cities. The insects will be collected by entomological nests and traps. Also, the plant species explored by Apidae (bees and bumblebees) will be identified by collecting pollen samples from insects and microscopic pollen examination. Based on this data we will estimate significance of balcony greenery in comparison to plants located at low altitudes. The second part of experiments will determine what are insects' limitations in high flights and if more attractive plants encourage them to fly higher. We will track frequency distribution of visits at given balcony heights by video recording.

Collected data will allow to estimate the importance of balconies' greenery for insects in the cities. In highly urbanized city areas, there are limited possibilities to increase plant abundance. The results of the proposed research will provide information that can be used to arrange better our balconies and terraces, supporting nature conservation in the cities. Additionally, such social programs involving citizens can be used for educational purposes to increase ecological awareness of the society. We believe that the project's results would catch attention of researchers in such fields as ecology, nature conservation, behavioural ecology and evolution.

Scientific goal of the project

Description of the problem to be solved: Insects play a relevant role in all ecosystems' functioning. Their existence and abundance are essential for many processes, like pollination, herbivory, detritivory and nutrient cycling (Young & Gratton 2014). Insects are also an important food source for higher trophic levels. One of the most important insects' function is pollination. About 80% of wild plants are estimated to depend on insects' pollination (Ollerton et al. 2011). What is more, insects' pollination cover even 30% of food resources consumed by humans (McGregor 2002). Nowadays, due to habitat loss and fragmentation, as well as general deterioration of habitat quality and global climate changes, we observe a rapid decline of insects' richness and biodiversity (Vanbergen & Initiative 2013; Hallman et al. 2017). Vulnerable insect habitats are progressively lost also through progressive urbanization (Fattorini 2011; Matteson et al. 2012). Not only are the areas of cities are expanding but also within the cities so far wild and vulnerable natural areas are paved over. In response, many city authorities try to undertake some actions to improve insects' conditions in large agglomerations. In more and more cities, roadside green belts are replaced with deliberately sown wildflower meadows. Parks and recreation places are arranged with vegetation supporting insects. Scientific studies

confirm that such campaigns significantly increase abundance and biodiversity of wild pollinators (Daniels et al. 2020; Mody et al. 2020), but further solutions are still needed.

Much is known about the impact of terrestrial vegetation on the pollinators, however nothing about the potential impact of balcony and terrace plants. Taking into account the high-density and mostly block of flat housing in cities, balconies and terraces provide a relevant usable area. Green and full of flowers balconies can not only be a great decoration of buildings but also fulfil important ecological functions. Studies have shown that generally people are eager to arrange their balcony with plants, but rarely consider its utilities for pollinators (Krzymińska et al. 2020). Proper management of these spaces, providing plants that can be a source of pollen and nectar, could significantly improve insects' condition in cities (Haaland et al. 2011; Samnegard et al. 2011). The results of the research proposed here will provide crucial information about the importance of good management of the balcony space, as well as can be used for environmental education and taking actions supporting nature conservation in the cities.

Within the project we aim to fill existing knowledge gap by **examining significance of balcony vegetation in the city areas for insects' abundance and biodiversity**. We will test following hypothesis:

Hypothesis 1 – City areas with enriched balcony vegetation increases pollinators abundance and biodiversity.

Hypothesis 2 – Pollinators collect pollen from plants located at balconies (higher than 2m above the ground)

Hypothesis 3 – Pollinators do not visit flowers located higher than 20 meters above the ground

Hypothesis 4 – More attractive plants lure pollinators for higher flights than less attractive plants

Significance of the project

State of art: Insects provide many ecological functions crucial for proper functioning of ecosystems. They are essential for processes such as pollination, detritivory, herbivory or nutrient cycling. Certainly, for people the most important function is pollination. About 80% of wild plants are estimated to depend on insects' pollination (Ollerton et al. 2011). What is more, insects cover even 30% food resources consumed by humans (McGregor 2002). The main group of pollinators are wild bees and bumblebees (Apoidea), butterflies (Lepidoptera), some flies (Syrphidae) and beetles (Coleoptera) (Rader et al. 2016). It is well documented that the abundance and biodiversity of pollinators are progressively decreasing. In the cities, authorities undertake variety of actions trying to protect them. Replacing roadside green belts with deliberately sown wildflower meadows brings positive effects (Mody et al. 2020), so even apparently small areas covered with plants can have a positive impact on the animal inhabitants of the cities. Arranging parks and recreation places with more insect-friendly greenery or extensive lawn mowing also plays an important role (Daniels et al. 2020). There are even attempts to create a vertical forest in the cities. The most famous example is Bosco Verticale in Milan, a 26 floors' skyscraper with balconies with trees, shrubs and climbing plants, including a variety of fruit bearing trees and flowering plants. Scientific research confirmed that such a solution has a positive effect on birds, which are willing to nest there. (Belcher et al. 2018). Therefore, balconies with properly arranged flowering greenery could also be additional areas that will support pollinators.

Justification for tracking proposed scientific problem: Efficient nature conservation is crucial nowadays. Rapidly decreasing abundance and biodiversity of insects is one of the major problems especially in highly transferred areas, such as big cities. However, in highly urbanized zones, there is a little possibility to increase plants abundance. Properly arranged, rich balcony vegetation can become a significant nutrient source for insects. Still, it has not been checked whether insects indeed explore plant nutrient sources located at higher altitudes. Within the proposed project we want to monitor dynamic of insects' abundance and biodiversity after significant increasement of balcony vegetation in representative research zones. Additionally, we will find out whether plants located at higher altitudes would become a significant source of pollen. Next, we will determine the maximum height of plants location, which are still explored by insects and whether this height is influenced by plants' attractiveness. Proposed research will fill existing knowledge gap in ecology and

entomology field as well as can have applicational relevance. The project has the potential to catch the attention of researchers in a number of biological fields, including entomology, conservation biology and animal behaviour, as well as to reach fields outside of the life sciences such as architecture and urban planning.

Scientific importance for development of research fields: Results of the first part of the research (testing hypothesis 1&2) will provide important information that can be used in subsequent studies about urban areas' management. After project completion, our research questions can be further investigated via extension to other urban spaces (such as rooftop areas, special terraces with attractive plants or modern buildings like skyscraper Bosco Verticale in Milan covered with vertical gardens). Conclusions from the second part (testing hypothesis 3&4) will make a significant contribution to the state of knowledge about food-gaining ability of pollinating insects. Knowledge of the insects' maximum flight heights and their potential ability to distinguish between profitable and less or not profitable flowers above the ground can be used in further studies in ecology, behavioural ecology, botany or even evolution fields (for example in research testing life strategies of various insects).

Social relevance: Apart from scientific significance, presented research will have applicational relevance. Management of green areas in cities is a challenging issue. Some actions, like sowing the flowery meadows or extensive mowing of lawns had already been proved to increase insects' abundance and biodiversity. Conclusions made based on our proposed research may become the next important source of information for governments and city authorities. Citizens eagerly grow plants on their balconies. If the most efficient way of balcony arrangements can be identified, it can become both an important action for nature conservation in cities and society educational programs increasing ecological awareness.

Concept and work plan

General work plan:

Part 1 - Testing hypothesis 1 & 2: The research zones would be located in two polish cities with number of citizens higher than 500 000 (Kraków and Warszawa). **Task 1** – Based on GIS data, we will choose 10 **research zones** of 1 km² (square area, 1 km x 1 km) in each city - five zones with high urbanization and five zones with low urbanization. We define high urbanization as an area where more than 50% of the surface is transferred, while low urbanization as an area where less than 25% of the surface is transferred. The two types of research zones will be used to compare whether varying terrestrial vegetation influence insects' eagerness to collect pollen from plants located at higher altitudes. **Task 2** – We will characterize the areas surrounding our chosen research zones. Within 5 kilometres from the research zone centre we will describe types of area including: green areas such as forests, meadows, parks and human-transferred areas such as large-scale buildings, estates or houses. **Task 3** – During the 1st year of the project (summer 2021) we will determine insects' abundance and biodiversity in all research zones, using traps. Additionally, we will collect Apidae (bees and bumblebees) by entomological nets to examine which plants species are the most attractive as a pollen source in given research zone. We will also carry out a detailed inventory of nature by documentation of all plant species already present in each research zone. **Task 4** – Within chosen research zones, in 2021, we would recruit volunteers, who are willing to participate in the project (during the whole project duration from 2021-2023) and agree for balcony arrangement. The chosen balconies cannot be located higher than the 3rd floor. The advertising campaign and questionnaire will be conducted *a priori* to estimate citizens willingness to participate in the project. **Task 5** - In spring 2022 we will arrange chosen balconies in all research zones. Within each 1 km² research zone we want to introduce additional plant surfaces of ~400 m², having ~1 m² of plant surface at each balcony (1 m² is an equivalent of 4 oblong flowerpots size 1 m x 0.25 m). We plan to use following plant species: *Lavendula sp.*, *Centaurea cyjanus*, *Thymulus serpyllum*, *Nepeta cataria*, *Echinacea purpurea*, *Ranunculs sp.* *Leucanthemum vulgare*, *Knautia arvensis* to arrange balconies.

The plant species had been chosen based on literature research. We use greenery that had been proven to be highly nectareous and pollen-bearing. **Task 6** – To verify **hypothesis 1** we will monitor the dynamics of insects' abundance and biodiversity in areas where we significantly increased plant vegetation at balconies. We will use traps set up 3 times during each summer season. **Task 7** – Using entomological nets, we will collect *Apidae specimens* 3 times during each summer season, to check whether insects explore pollen sources arranged at the balconies (**hypothesis 2**). Plant species will be identified by microscopic examination of pollen. **Task 8** – As some of the chosen plants are annual and we also predict that not all volunteers would grow plants correctly, we plan to replace some greenery, so that the balconies' arrangement during summer 2023 would be similar to the year before.

Part 2 - Testing hypothesis 3 & 4: The experiment to verify hypothesis 3 and 4 would be performed in 2023. Arranged balconies would be located between 1st and 10th floors of blocks of flats. **Task 9** - We will choose 4 estates to set up experimental arrangement of balconies. The estates would be located in Kraków and have moderate level of urbanization (~30% of transferred area). The advertising campaign and questionnaire will be conducted *a priori* to estimate citizens willingness to participate in the project. Within each estate, we want to arrange all balconies in four blocks of flats. Chosen four blocks of flats need to have the same building plan and at least 10 floors high. Each block of flats would have balconies arranged differently. **Arrangement 1** - All balconies in one vertical segment are arranged with ~2 m² of **highly attractive plants** (*Lavendula sp.*, *Centaurea cyjanus*, *Thymulus serpyllum*, *Nepeta cataria*, *Echinacea purpurea*, *Ranunculs sp.*, *Leucanthemum vulgare*, *Knautia arvensis*). - Fig. 1 A1. **Arrangement 2** – All balconies in one vertical segment are arranged with ~2 m² of **less attractive plants** (*Begonia serpensflores*, *Petunia sp.*, *Tagetes panula*, *Tagetes tenuifolia*, *Tagetes erecta*, *Fuchsia sp.*, *Dahlia sp.*, *Lobelia erinus*, *Lobularia maritima*). - Fig. 1 A2. **Arrangement 3** – Only balconies at given floor (height) in all segments are arranged with ~2 m² of highly attractive plants (the same species composition as in arrangement 1). - Fig. 1 A3. **Arrangement 4** – Only balconies at given floor (height) in all segments are arranged with ~2 m² of less attractive plants (the same species composition as in arrangement 2). - Fig. 1 A4. The arrangements 1 and 2 will be fixed during whole spring and summer season. The floors for arrangements 3 and 4 will be changed weekly in random order. Highly and less attractive plants will be analysed as categorical binary scale. Plant species had been chosen based on literature review, and we divide them into categories based on their pollen and nectar yield. **Task 10 – Data collection** – For arrangements 1 and 2 we will record 3 weeks - one week per month in June, July and August. For arrangements 3 and 4 we will record a whole week with plant arranged at given heights but analyse only one day with optimal weather conditions (sunny, warm day, without rain). All video records will be taken during daytime.

We would like to ensure that experiment setup with cameras would not invade privacy of volunteers. Cameras would be pointed into flowerpots and will not include in the frame any inside parts of volunteers' flats.

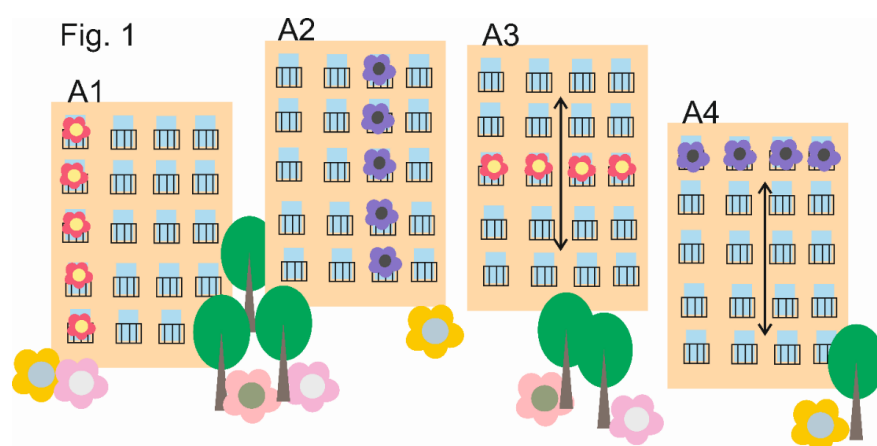


Figure 1. Schematic representation of planned balconies' arrangements for part 2.

Research goals: **Goal 1:** We aim to describe dynamics of species abundance and biodiversity in research zones during project duration – before balcony arrangement (summer 2021), in first year with arranged balconies (summer 2022), in second year with arranged balconies (summer 2023). **Goal 2:** We want to learn whether plants located at higher altitudes (more than 2 meters) can be a significant pollen source for bees and bumblebees. **Goal 3:** We will establish maximum altitude of plant location at which plant are visited. Completing the goal will allow the hypothesis 3 to be verified. **Goal 4:** We want to determine whether pollinators are more eager to fly higher to reach more attractive plants.

Risk analysis: We estimate overall risk of the project as moderate. We take into account that citizens might not be willing to participate in the project and allow for their balconies' arrangement and monitoring. However, previous campaign and questionnaires would rise ecological awareness and highlight importance of the project. Balconies' arrangements would be free of charge for participants and attractively prepared by a professional company. What is more, additional greenery on chosen balconies in part 2 of the research would be very rich, so in case if some residents wouldn't agree to have their balconies empty /arranged they would not significantly disturb the results. Involvement of the volunteers introduces also a possibility that some plants would be grown incorrectly. We will provide information leaflets for volunteers to reduce risk of plant death, as well as the possibility to contact hired gardening company. Additionally, we included in the budget costs for replacement of 60% of plants in part 1 for second experimental season (summer 2023). As the project involve field observations, weather conditions may also force changes in the schedule. Extra time had been booked for such possibility. Other parts of the project are at very low risk. Insect and pollen species collection and identification are standardized methods, which are also frequently used by our research team. We also have experience in video data analysis.

Research methodology

Research zones: For testing hypothesis 1 and 2 we will choose 20 research zones in two big polish cities (10 research zones in each), sized 1 km x 1 km. Half of the zones would be highly urbanized, and latter half would be with low urbanization. The zones would be chosen based on publicly available GIS data of the cities, where the surface is divided into green and transferred areas. We define high urbanization as an area where more than 50% of the surface is transferred, while low urbanization as an area where less than 25% of the surface is transferred. For part 2 of the project (testing hypothesis 3 & 4) we will use GIS data to determine surface urbanization of chosen estates. In both parts we will conduct an information campaign and questionnaire to estimate residents' willingness to participate in the project.

Data collection: Entomological nets – Using entomological nets is a common method to collect flying insects. We will use them to complete tasks 3 and 7. Caught insects would be immediately placed into separate tubes, so that pollen samples could be analysed later under a microscope. Both insects and plant species would be identified. **Traps** – We will locate traps on the ground level, and they will be used to complete tasks 3 and 6. Caught insects' species would be identified later in the laboratory. Based on that information we will calculate what percentage of pollen come from greenery arranged at balconies, in comparison to plants located at lower altitudes. **Cameras** – Videos will be used to complete task 10. We will identify insects' orders which appear on recordings. We will analyse insects' visits' frequency distribution at given height of balconies.

Balconies' description: We will measure parameters describing environmental conditions of each balcony, including its' directional exposure, average prevailing winds and temperature. These data would allow to distinguish if observed insects' visit frequency is indeed caused by experimental manipulation.

Data analysis: All statistical analyses will be performed in the R software (R Core Team) using standard statistical tests. For testing hypothesis 1 and 2, we will statistically analyse dynamic of insects' species abundance and biodiversity before experimental treatment (balconies arrangement), during first and the

following year after balconies arrangements. For testing hypothesis 3 & 4 we will determine significant differences between abundance of insects at chosen heights of balconies.

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Table with budget of the project

PROPOSED BUDGET

No	Item	Funds for each budget year (PLN)			
		2021	2022	2023	Total
1.	Direct costs, including:	144 000,00	549 000,00	382 520,00	1 075 520,00
	- Salaries and scholarships	60 000,00	60 000,00	60 000,00	180 000,00
	- Equipment and software	30 000,00	60 000,00	-	90 000,00
	- Other direct costs	54 000,00	429 000,00	322 520,00	805 520,00
2.	Indirect costs (20% of direct costs)	28 800,00	109 800,00	76 504,00	215 104,00
	Total costs (1+2)	1 728 000,00	658 800,00	459 024,00	1 290 624,00

Breakdown of project costs including justification and relevance for the tasks in the project

Salaries and scholarships:

1. Salary for two PI's: $3 \text{ years} \times 12 \text{ months} \times 1\,500 \text{ PLN} \times 2 \text{ PIs} = 108\,000 \text{ PLN}$
Scope of work: Experiment planning and tasks coordination, people management, supervision and training data analysis and interpretation, preparation of manuscripts
2. Scholarships for Master or Bachelor Students: $3 \text{ years} \times 12 \text{ months} \times 1\,000 \text{ PLN} = 72\,000 \text{ PLN}$
Scope of work: field work, sample collection and laboratory analysis

Equipment and software

1. Laboratory and field equipment (such as entomological nets and traps, laboratory plastic): 20 000 PLN
2. Cameras: $30 \text{ cameras} \times 2\,000 \text{ PLN} = 60\,000 \text{ PLN}$
3. Laptops: $2 \text{ laptops} \times 5\,000 \text{ PLN} = 10\,000 \text{ PLN}$

Other direct costs

1. Balconies arrangements: Total: 623 520 PLN
 - For testing hypothesis 1&2: $2 \text{ cities} \times 10 \text{ research zones} \times 400 \text{ m}^2 \times 4 \text{ flowerpots (per } 1\text{m}^2) \times 10 \text{ PLN (cost of one flowerpot) + 60\% for plant replacement in next year} = 512\,000 \text{ PLN}$
 - For testing hypothesis 3&4: $4 \text{ estates} \times (10 \text{ balconies (arrangement 1)} + 10 \text{ balconies (arrangement 2)} + 5 \text{ balconies (arrangement 3)} + 5 \text{ balconies (arrangement 3)}) \times 8 \text{ flowerpots (per balcony)} \times 10 \text{ PLN} + 20\% \text{ for plant replacement} = 11\,520 \text{ PLN}$
 - Planning and execution of balconies arrangements by external company: 100 000 PLN
2. Preparation of advertising materials – 10 000 PLN
3. Preparation of educational leaflets for volunteers who would agree for balcony arrangement - including graphical services, printing and distribution – 10 000 PLN
4. Travel costs – 12 000 PLN
5. Scientific conferences – including travel costs, accommodation and conference fees – $5\,000 \text{ PLN per conference} \times 2 \text{ conferences} \times 4 \text{ people (2 PIs and 2 Students)} = 40\,000 \text{ PLN}$
6. Publication costs – including linguistic proofreading and open access fee – 10 000 PLN
7. Other outsourcing (covering costs such as poster prints, graphical services, linguistic correction) - 10 000 PLN
8. Ten people for field support in high research seasons – $3 \text{ months employment} \times 3 \text{ years} \times 10 \text{ people} \times 1\,000 \text{ PLN} = 90\,000 \text{ PLN}$
Scope of work: recruiting of volunteers, collecting data from cameras/traps, supervision of balconies during summer season.

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