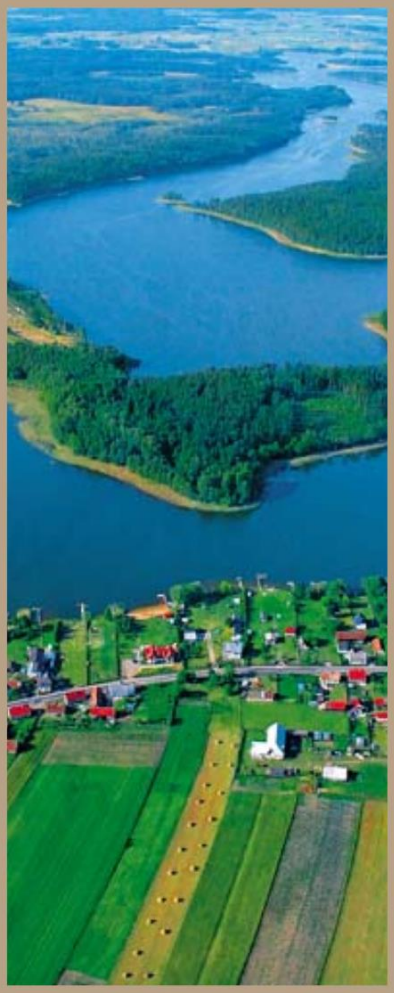
Institute of Environmental Sciences
Jagiellonian University

# GRANT WRITING IN ENVIRONMENTAL STUDIES

*Compendium of students' projects 2020/21*





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## About the course

The aim of the course is to give students practical experience with writing project in the field of environmental sciences. The course consists of 10 meetings, each lasting 3 hours. During the meetings, two teachers give conservatory lectures and guide participant in the process of writing project. Students come up with their own ideas, form groups and write proposals on the topic of their own interests. The proposal can either be hypothesis-driven research project or project aiming at nature conservation. The projects are written according to the British Ecological Society grant scheme (https://www.britishecologicalsociety.org/funding/research-grants/), with some modifications. Proposals are evaluated by external reviewers and by the fellow students. They are corrected and defended in front of the audience. Proposal included in the current compendium are the final versions of projects (excluding CVs of the applicants).

The final score is based on timeliness and formal and substantive correctness of the assignments.
Scale of final grade (5 to 2 scale and corresponding A – F scale):

5.0 (A): All written assignments completed according to the guidelines and deadlines, active participation in the discussions.

4.5 (B): All written assignments completed according to the guidelines and deadlines.

4.0 (C): Written assignments completed according to the deadlines, with some errors in substantive correctness

3.5 (D): Written assignments completed according to the deadlines, with major errors in substantive correctness

3.0 (E): Written assignments competed past the deadlines

2.0 (F): Failed to meet the minimal requirements, i.e. attendance in at least 24 h of classes and completed assignments.

The course is facultative, has limit of 15 participants, and thus is recommend only for highly motivated students. Good command of English is required. Team work is fun!

# Teachers, Reviewers & Students

**Teachers**

Dr hab. Joanna Rutkowska, prof. UJ – Institute of Environmental Sciences, Jagiellonian University

Dr hab. Taduesz Zając, prof. IO PAN- Institute of Nature Conservation, Polish Academy of Sciences

**Reviewers**

Dr hab. Paweł Adamski, prof. IO PAN - Institute of Nature Conservation, Polish Academy of Sciences

Dr Adam Ćmiel - Institute of Nature Conservation, Polish Academy of Sciences

Dr Agnieszka Gudowska - Institute of Environmental Sciences, Jagiellonian University

Dr Wojciech Solarz – Institute of Nature Conservation, Polish Academy of Sciences

**Students**

Alina Bondur – II year of Ecology and Evolution

Mateusz Chechetkin – II year of Ecology and Evolution

Miao Dang – I year of Enviromental Protectiton and Managment

Luis Eyzaguirre Rico – II year of Ecology and Evolution

Magdalena Honkowicz – II year of Ecology and Evolution

Kamila Kadyrova – I year of Enviromental Protectiton and Managment

Evy Laa – II year of Ecology and Evolution

Bagas Prabovo – I year of Enviromental Protectiton and Managment

Agata Różańska – II year of Ecology and Evolution

Vrinda Sharma – I year of Enviromental Protectiton and Managment

Febrina Siahaan – II year of Ecology and Evolution

Karolina Sorys – II year of Ecology and Evolution

Małgorzata Śliż – II year of Ecology and Evolution

Dalexis Tolosa Arias – II year of Ecology and Evolution

Maryellen Zbrozek – I year of Enviromental Protectiton and Managment

Jiahui Zhang– II year of Ecology and Evolution

## Topics for projects suggested by the course participants

**Voting carried out during the class identified the most popular topics (marked with ticks)**

- [x] A comparative study of tea and coffee on improving the excitability of animals (white mice)
- [ ] Research on vegetarians that are healthier than normal dieters (including meat and vegetables)
- [ ] Characteristics of by-products of tannic acid under chloramine disinfection conditions
- [ ] Spatial-temporal distribution and types of microplastics in inland waters
- [x] Analysis of Nitrosoamines on Baltic Sea fish
- [ ] Identifying Positive and Negative impacts of traditional Indian outdoor air purifying techniques
- [ ] Fate of microplastic pollutant during wastewater treatment in different WWTP's in Poland
- [ ] Usage of advanced irrigation technologies to fight drought consequences in Poland
- [ ] Trade of pets and meat as a driver of the spread of the pathogenic chytrid fungi around amphibians' population
- [x] Waste management of temple floral offerings in Bali
- [ ] Effect of wildfires on the inoculation and distribution of mycorrhizae in the tropical rainforest
- [ ] Effect of triclosan toxicity on activated sludge activity, based on the viability of Lecane inermis rotifers
- [ ] Effect of endophytic yeast on the inhibition of pathogenic fungus development, as exemplified by wheat growth
- [x] Do the insect houses really increase amount of insects?
- [x] Different surface influences the vibrations produced by spiders
- [ ] Mollusks as potential bioindicators of heavy metal contamination in the coastal area of Sopot, as a consequence of tourist over-exploitation
- [ ] Bioaccumulation of heavy metals in hyperaccumulative plants as a possible phytoremediation treatment in mining areas in Krakow
- [ ] Investigation of the environmental impact of antimony from mining waste
- [x] Bioleaching of antimony from mining waste
- [x] Effects of Rat Maternal Behaviour on Reproductive Success in Male Offspring
- [x] Experimental evolution of social vs solitary subtypes in *C. elegans* under selective pressure of prey scarcity
- [ ] Whether bank vole females selectively prefer «fitter» males to control ones for future mating?
- [ ] Do bank vole females previous pregnancy experiences correlate with their postpartum mating behavior?
- [x] Evolutionary Mechanism of Variation Leaf Veins Traits in Relation with Global Climate Change
- [ ] Distribution, population size of the Vulnerable species Hopea sangal in Indonesia
- [ ] Interspecies transmission of Nosema ceranae in wild pollinator communities through shared floral resources
- [x] Eutrophic lakes: Are microplastics from agricultural runoff substrates for algal growth?
- [ ] Impact of fire on invasive species and host plants of Lycaenidae butterflies in Dębnicko-Tyniecki Meadow Area (Natura 2000)
- [x] Do feathers age? Possible changes in flight feathers' mass and growth rate in aging birds
- [x] Application of Agroforestry in Oil Palm Plantations
- [ ] Ecological Potential of Home garden Agroforestry in Banyumas Regency
- [ ] Research on plastic pollution on the Baltic area
- [x] Conservation efforts to protect bats from societal misconceptions

# 1. Microplastics from agricultural runoff as potential substrates for algal growth in eutrophic lakes

## Applicants names

Maryellen Zbrozek, Kamila Kadyrova, Vrinda Sharma, Bagas Adji Prabowo

## Abstract

Eutrophication is a significant problem affecting the proper functioning of aquatic ecosystems. Mitigating its negative effects requires a complex understanding of its causes. It is widely known that agricultural runoff containing high concentrations of phosphorus and nitrogen is usually the main driver of eutrophication in freshwater lakes. Agricultural runoff has also been shown to contain microplastic particles in addition to nutrients. Recently, multiple studies have shown that microplastic may act as a substrate for algal growth. Since algal growth is the main effect of eutrophication in freshwater lakes, this leaves one unanswered question: Are microplastics another significant component of agricultural runoff that contributes to lake eutrophication? This study will examine the extent to which microplastic is present in agricultural runoff and the extent to which it is present in connected eutrophic lakes in northern and western Poland. Furthermore, this study will examine the relationship between microplastic concentration and algae growth rate under controlled nutrient levels in laboratory conditions. This project will provide insight into a potential driver of high levels of lake eutrophication and may be able to provide a basis for future agricultural practices and policies to reduce eutrophication by regulating the amount of microplastic running into lakes.

## Summary of Project Details

| | |
|---|---|
| **Total project cost:** | 587,221 PLN |
| **Project title:** | Microplastics from agricultural runoff as potential substrates for algal growth in eutrophic lakes |
| **Project start date:** | 01/03/2021 |
| **Project end date:** | 31/12/2022 |
| **Project country:** | Poland |
| **Up to 6 key-words:** | Microplastic; agricultural runoff; algal blooms; eutrophic lakes |
| **Project lay summary:** | Harmful algal blooms are a major threat to aquatic ecosystems. It is widely known that increased levels of algae growth are caused by nutrients from agricultural fields running into nearby bodies of water. Microplastic, an increasingly common artificial substance in natural environments, has recently been shown to be an item on which algae can grow. Since microplastic has been found in runoff from agricultural fields, agricultural practices may be causing microplastic to end up in nearby lakes. This project aims to determine whether microplastic originating from agriculture is an additional cause of harmful algal bloom growth in lakes. |

## Project description

**Core project description**

Eutrophication, the process of algal blooms growing on water bodies due to the excessive input of nutrients such as phosphorus and nitrogen, has been an increasing problem in lakes for nearly a century, largely due to human agricultural activity (Schindler et al., 2016; Hasler, 1947). It has been noted as the planet's most widespread water quality problem, causing fish to die, ecological imbalance, deterioration of water quality, and health problems if the water is consumed (Bhagowati & Ahamad, 2018; Schindler et al., 2016). As the growing world population depends heavily on large-scale agriculture reliant on heavy fertilizer use, eutrophication is likely to continue to be a problem. Multiple studies have shown that decreasing the amount of nitrogen input into lakes does not mitigate the effects of eutrophication, although reducing phosphorus input can (Schindler, 2012; Schindler et al., 2008). However, modern agriculture depends on both nutrients, so this does not bode well for reducing eutrophication. Unfortunately, eutrophication of freshwater is a significant driver of methane emissions, and the expected acceleration of eutrophication in the 21$^{st}$ century is expected to increase methane emissions by 30-90% over the next century, the equivalent to 18-33% of annual $CO_2$ emissions from the burning of fossil fuels (Beaulieu, DelSontro, & Downing, 2019).

Like many industries in the modern age, the agricultural industry is becoming increasingly reliant on plastic. For example, plastic pipes are frequently used for irrigation, waste containing microplastics is used as fertilizer, and many types of mulch contain plastic (He et al., 2018). Plastic breaks down into small particles called microplastics, which are a widely recognized problem in aquatic environments. Such microplastics have been found in agricultural runoff (Grbic et al., 2020), likely stemming from the agricultural industry's use of plastic products. Agricultural runoff is thus likely to contain both eutrophication-causing nutrients and microplastic particles.

Colonization of microplastic particles by microalgae and cyanobacteria has been described in marine ecosystems (Yokota et al., 2017; Reisser et al., 2014). Although fewer studies have been conducted in freshwater ecosystems, Canniff & Hoang (2018) found that the alga *Raphidocelis subcapitata* has a higher growth rate when exposed to plastic microbeads in freshwater, and Arias-Andres et al. (2018) found biofilms of colonized green algae on microplastic particles in three temperate lakes. These studies suggest that microplastics could serve as substrates for algae growth.

Considering the co-occurrence of nutrients and microplastics in agricultural runoff, we intend to explore whether microplastic, which serves as a substrate for algal growth, can act as an additional driver of eutrophication in lakes affected by agricultural runoff. We will test four hypotheses: (1) Microplastics from agricultural runoff are transferred to adjacent eutrophic lakes, (2) Lakes with higher levels of eutrophication will have higher concentrations of microplastics compared to lakes with lower eutrophication levels, (3) Microplastics in simulated agricultural runoff conditions have a positive effect on algae growth, and (4) The growth rate of algae on microplastics is higher compared to the growth rate of algae on natural substrate (silt) under simulated agricultural runoff conditions. If our study is successful, this knowledge could be critical for understanding and mitigating the effects of agriculture on eutrophication in lakes.

**Methods**

*Field Sampling*

We will select 20 agricultural lakes near agricultural fields in northern and western Poland, where there is a large agricultural economy and where lake eutrophication has been frequently observed (Kowalczewska-Madura et al., 2019). We will select lakes with few other plastic sources nearby. We will be split into four teams with one principal investigator as the leader and one field assistant per team. Two teams will work in northern and two teams in western Poland region. Sampling will be conducted during peak agricultural periods, with each team working simultaneously from June to August. Selected lakes will be of similar sizes, ranging from 500-2000 $m^2$. Depending on the lakes' sizes, each will be divided into 15 evenly distributed sampling plots, from which one water sample will be taken randomly. This process will be replicated three times, once per month. Lake water will be sampled via wading or by boat. The water samples will be collected in line with the methodology described by Musselman in 2012. At each sampling plot, the dissolved oxygen (DO) concentration (mg/L) will be measured using a calibrated dissolved oxygen sensor. Each sample will be taken from the photic layer of the water, which will be determined via a secchi disk. Each sample will be placed in a 100 mL plastic tube and stored for later analysis.

At each lake, all sources of agricultural runoff will be identified. Twenty water samples will be collected from each runoff source. Runoff samples will be collected from plots at evenly distributed intervals spanning from the lake to the point upstream at which agricultural activity is taking place. Each sample will be placed in a 100 mL plastic tube and stored for later analysis.

Lake water samples will be analysed in the laboratory to measure the overall level of eutrophication and the average concentration of microplastics present in each lake. Additionally, we will conduct a meta-analysis to compare and differentiate the plastic sources we find. Level of eutrophication will be measured by analysis of three factors: DO concentration (measured in the field), chlorophyll-*a* concentration, and nutrient concentration (phosphorus and nitrogen). Chlorophyll-*a* concentration (ug/L) will be measured via spectrophotometry after the water samples have been filtered, dried, and dissolved in acetone. Concentrations of phosphorus and nitrogen in each water sample will also be measured via spectrophotometry and reported in units of ug/L.

To measure microplastic concentrations, water samples will be run through a stacked sieve system, with a 5 mm mesh sieve to remove larger particles on top of a 0.05 mm mesh sieve to collect finer particles including microplastics. Any clearly non-plastic particles will be removed. The material on the 0.05 mm sieve will be rinsed with distilled water. Collected material will be transferred to glass beakers and dried in a 90 °C drying oven for 24 h. To remove organic material, wet peroxide oxidation (WPO) will be conducted by adding aqueous 0.05 M Fe(II) solution to the beaker, followed by adding 30% hydrogen peroxide. WPO solution will be run through a density separator and air dried. The resulting microplastic particles will be weighed to find the concentration of microplastics (ug/mL) per each sample. This process will be conducted for each sample collected from agricultural runoff, and the concentrations will be averaged for each runoff source. Microplastic concentration of lake water will similarly be measured.

*Laboratory Experiment*

The laboratory experiments will determine how microplastics affect algae growth under a controlled nutrient level. The observed concentrations of phosphorus and nitrogen in the lakes will be used to create a

simulated agricultural runoff solution that mimics that found within the natural study system. Microplastics collected during the field sampling portion of the experiment will be analysed and similar types of plastic will be purchased for use within the laboratory experiment in order to ensure that the microplastics we are testing will correspond to those found within the natural study system.

A total of 180 testing chambers will be prepared using freshwater, algae (*Scenedesmus obliquus*), agricultural runoff solution, and various concentrations of substrate in the form of either microplastic or silt. A total of ninety chambers will be used for each substrate type, with six varying substrate concentrations replicated fifteen times (Table 1). After 30 days, the algae will be collected, dried for 24 h, and the dry weight will be measured.

**Table 1:** Proposed experimental setup. The experiment will be replicated 15 times for a total of 90 observations for each substrate type.

| **Substrate** | | **Microplastic** | **Silt** |
|---|---|---|---|
| **Concentration** | 0 ug/ml | $m_0$ | $s_0$ |
| | 20 ug/ml | $m_{20}$ | $s_{20}$ |
| | 40 ug/ml | $m_{40}$ | $s_{40}$ |
| | 60 ug/ml | $m_{60}$ | $s_{60}$ |
| | 80 ug/ml | $m_{80}$ | $s_{80}$ |
| | 100 ug/ml | $m_{100}$ | $s_{100}$ |

We will build a mixed ANOVA statistical model in R software to analyse the results of both sampling and experimental part.

**Expected Outputs**

From the field sampling, we expect that microplastic will be present in both agricultural runoff and eutrophic lakes. We expect that the laboratory experiments will show increased rates of algae growth when presented with microplastic as a substrate, as opposed to silt as a natural substrate. These results would suggest that microplastic may be a previously unknown component of agricultural runoff that contributes to eutrophication in lakes.

We plan to share our findings at two scientific conferences, either through poster or lecture presentation. During one conference, we will focus mostly on the conclusions drawn from the field sampling analysis, while at the other we will emphasize the conclusions from the laboratory experiment results. We also plan to publish a manuscript with the findings of our study in a high-impact, open access journal.

**Project Timeline**

We estimate the total duration of the project to be one year and ten months, with the start date on 1 March 2021 (Figure 1). During the three months of preparatory work, we will purchase all the required equipment and consumables, hire assistants, pass the safety training, and conduct a meta-analysis based on

available studies in the area. Sample collection is planned for the summer period. Upon its completion, we will transfer the results of samples to the lab and start analysing it. The analytical phase is estimated to be the longest (September 2021-August 2022). We would like to commence the lab experimental phase in December 2021, when some analytics from the field will be ready. The estimated length of the experimental phase is three months.

We expect to start concluding the project and drafting the report at the final stage of the analytical phase, i.e. in June 2022. The report will be finalised by the end of November 2022. We will attend 2 conferences in December 2022 to share the results of our study with the scientific community, as described above. If the COVID-19 situation will inhibit our work, we will conduct the experiment with the same timeline in 2022.

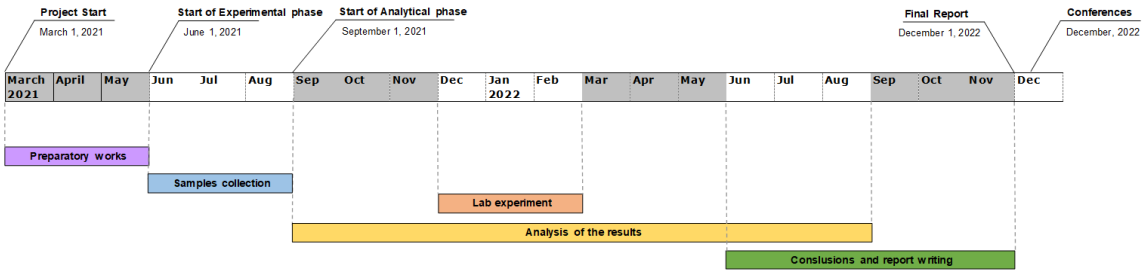


**Figure 1:** Estimated project timeline

**Additional questions:**

**What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?**

All team members will be required to have a preliminary occupational medicine examination prior to working on the project. We will ensure availability of driving licences for all people driving the vans. The drivers will follow Traffic Laws during the whole duration of the trip to ensure road safety.

During the field experiment we might face the following risks:

1. Lakes' banks failure. This will be minimised by inspecting the banks prior to starting sampling and selecting the banks with the most stable structure (least amount of erosion).
2. Water contamination. This will be minimized by wearing rubber gloves at all times of contact with water.
3. Falling into water, drowning. This will be minimised by wearing life vests at all times of the boats' use.

Additionally, we will carry a sufficient amount of water for each crew member and a first aid kit during each day of the field sampling.

We expect the lab risks to be solely related to safety hazards (e.g. usage of solvents, potential exposures to allergens, and physical hazards like slips and falls). This will be minimised by following the safety rules and lab standards, as applicable. Appropriate clothing, protective gloves, and eye wear will be worn at all times of performing the lab experiment.

**Please provide details of agreed collaborations and project partners that will facilitate the proposed project:**

The project will be realized in cooperation with the Jagiellonian University in Krakow (Department of Biology). During the experimental phase, the University lab facilities will be used.

**Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:**

UJ has a system of well-equipped and modern laboratories for environmental studies. We are planning to conduct our experiment in the Laboratory of Ecochemistry and Ecotoxicology, which has the necessary facilities and which will supply us with the necessary equipment (such as an elementary analyser CHNOS (Elementar Vario EL III), driers). The consumables and unavailable equipment (calibrated dissolved oxygen sensor, portable spectrophotometer, etc.) will be purchased separately under the proposed budget.

**Please provide details of necessary permits/licences obtained, if applicable:**

We will contact Polish local authorities (e.g. Urząd Gminy) to find out if a permit is required to access each particular lake. We will request for any necessary permits upon receipt of the authorities' confirmation.

**Please indicate how you will assess the scientific impact of the project and the benefits for non-academic audiences:**

We expect that the research results will be of high practical importance to the local farming community, as it shows the effects of microplastic on agricultural runoff and eutrophication in adjacent lakes. Equally, we believe it will be useful to policy makers in the agricultural field, as it will provide insights into possible ways to improve the existing laws applicable to microplastic use.

**How do you intend to make your research data publicly available?**

We plan to publish a manuscript with the findings of our study in a high-impact, open access journal. We will also present the results of the study at two scientific conferences, as described above.

**Please provide details of any published papers relevant to this project:**

Arias-Andres, M., Kettner, M. T., Miki, T., & Grossart, H. P. (2018). Microplastics: New substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems. *Science of the Total Environment*, *635*, 1152-1159.

Beaulieu, J. J., DelSontro, T., & Downing, J. A. (2019). Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. *Nature communications*, *10*(1), 1-5.

Bhagowati, B., & Ahamad, K. U. (2019). A review on lake eutrophication dynamics and recent developments in lake modeling. *Ecohydrology & Hydrobiology*, *19*(1), 155-166.

Canniff, P. M., & Hoang, T. C. (2018). Microplastic ingestion by Daphnia magna and its enhancement on algal growth. *Science of the Total Environment*, *633*, 500-507.

Grbić, J., Helm, P., Athey, S., & Rochman, C. M. (2020). Microplastics entering northwestern Lake Ontario are diverse and linked to urban sources. *Water Research*, *174*, 115623.

Hasler, A. D. (1947). Eutrophication of lakes by domestic drainage. *Ecology*, *28*(4), 383-395.

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., & Lei, L. (2018). Microplastics in soils: analytical methods, pollution characteristics and ecological risks. *TrAC Trends in Analytical Chemistry*, *109*, 163-172.

Kowalczewska-Madura, K., Dondajewska, R., & Gołdyn, R. (2020). Internal phosphorus loading in eutrophic lakes in Western Poland. In *Polish River Basins and Lakes–Part I* (pp. 277-303). Springer, Cham.

Musselman, R. (2012). Sampling procedure for lake or stream surface water chemistry. Res. Note RMRS-RN-49. *Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 11 p*

Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D. K., Thums, M., ... & Pattiaratchi, C. (2014). Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. *PloS one*, *9*(6), e100289.

Schindler, D. W. (2012). The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1746), 4322-4333.

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success.

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., ... & Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences*, *105*(32), 11254-11258.

Yokota, K., Waterfield, H., Hastings, C., Davidson, E., Kwietniewski, E., & Wells, B. (2017). Finding the missing piece of the aquatic plastic pollution puzzle: interaction between primary producers and microplastics. *Limnology and Oceanography Letters*, *2*(4), 91-104.

## Budget

Equipment

| Item | Quantity | Description | Total Cost, PLN |
|---|---|---|---|
| GPS | 2 | One for each region, for navigation | 800 |
| Laptops | 4 | One for each team | 8,000 |
| Buckets | 12 | Used for sampling lake water; three for each team | 48 |
| Sample storage equipment | 10 | For storing samples in the field and for transport | 300 |
| Field Phones | 4 | One for each team; for safety | 2,000 |
| Secchi disk | 4 | To measure the photic level of the water; one for each team | 1,360 |
| Dissolved oxygen sensors | 4 | One for each team | 4,800 |
| Portable spectrophotometer | 4 | To measure nitrogen and phosphorus in the field; one for each team | 14,400 |
| Glass beakers | 60 | For drying of samples | 450 |
| 5 mm mesh sieve | 5 | Large sieve to remove large particles | 600 |
| 0.5 mm mesh sieve | 7 | Small sieve to collect microplastics | 1,750 |
| Glass growth chambers | 60 | For laboratory experiments, three rounds of growth periods will be used | 6,000 |
| Overall Equipment Cost: | | | 40,508 |

Consumables

| Item | Quantity | Description | Total Cost, PLN |
|---|---|---|---|
| 100 ml plastic tubes | 1200 | For containing water samples | 4,800 |
| Glass Fiber filters | 10 | 100pcs/pack; for processing samples to measure chlorophyll-*a* | 2,000 |
| Acetone | 2 | Solvent for measuring chlorophyll-*a* | 70 |
| Algae | 5 | For use in growth rate experiments | 2,400 |
| Silt | 2 | Natural substrate | 160 |
| Iron (II) sulphate*7H20 | 2 | For WPO during microplastic analysis | 204 |
| Hydrogen Peroxide 30% | 2 | For WPO during microplastic analysis | 536 |

| Item | Quantity | Description | Total Cost, PLN |
|---|---|---|---|
| | | | |
| Gloves | 3 | 100 gloves/pack; field and lab work | 180 |
| Goggles | 8 | For laboratory work | 200 |
| Microplastics | 3 | 10 g packages of plastic microspheres | 1,163 |
| Overall Consumables Cost: | | | 11,713 |

Personal and Field Travel/Accommodation/Subsistence

| Description | Total Cost, PLN |
|---|---|
| Van Rental (for the field sampling duration) | 57,600 |
| Accommodation (renting 1 flat in the North and 1 flat in the West region) | 27,000 |
| Fuel (for vans during sampling period) | 12,000 |
| Boats (one for each team; plastic boat) | 5,200 |
| Life Vests (1 per person) | 800 |
| Outdoor Clothes | 5,000 |
| First Aid Kits (4 items) | 400 |
| Food (100 PLN/person/day for 90 days) | 72,000 |
| ICWRSD 2022: International Conference on Water Resources Sustainable Development, Tokyo (Dec 02-03, 2022) | 9,000 |
| ICEBESE 2022: International Conference on Environmental, Biological, Ecological, Sciences and Engineering, New York (Dec 9-10, 2022) | 10,000 |
| Publication cost in open access journals | 12,000 |
| Overall Travel Cost: | 211,000 |

Employment (note only casual, short term assistance will be considered)

| Position | Description of role | Rate, PLN & Duration of employment | Total Cost, PLN |
|---|---|---|---|
| Principal Investigator | Team leader (1) North Poland<br>Collection and analysis of samples | 3,000/month for 22 months | 66,000 |
| Principal Investigator | Team leader (2) North Poland<br>Collection and analysis of samples | 3,000/month for 22 months | 66,000 |
| Principal Investigator | Team leader (3) West Poland<br>Collection and analysis of samples | 3,000/month for 22 months | 66,000 |
| Principal Investigator | Team leader (4) West Poland<br>Collection and analysis of samples | 3,000/month for 22 months | 66,000 |
| Field Assistant / Lab Technician | Help taking samples<br>Transportation of samples<br>Help analyse samples | 1,000/month for 22 months | 22,000 |
| Field Assistant / Lab | Help taking samples | 1,000/months for 22 | 22,000 |

| Position | Description of role | Rate, PLN & Duration of employment | Total Cost, PLN |
|---|---|---|---|
| Technician | Transportation of samples<br>Help analyse samples | months | |
| Field Assistant | Help taking samples<br>Transportation of samples | 1,000/months for 8 months | 8,000 |
| Field Assistant | Help taking samples<br>Transportation of samples | 1,000/months for 8 months | 8,000 |
| Overall Employment Cost: | | | 324,000 |

| | |
|---|---|
| Total project cost | 587,221 |

# 2. Conservation Efforts to Protect Bats from Societal Misconceptions

## Applicants names

Dalexis Tolosa Arias, Evy Laa, Febrina Siahaan

## Abstract

There are so many myths about bats in Asia, which are threatening conservation. These myths lead to the misunderstanding and undervalue the position of bats among other animals. After the pandemic outbreak, there was a surge of news in the media mentioning that many local towns across different countries are trying to get rid of bats by killing or expelling them from their habitats. Even though this information was easily found through the media, little effort has been put into research on how the effect of the current pandemic is affecting the human-bat relationship. This project is making use of surveys in 4 different countries in Asia, comparing friendliness of bats and certain animals before and after knowing they are potential COVID carriers. The criteria for the destinations chosen would be given by levels of urbanisation and proximity to bats conservation locations. Lastly, after the conflict mapping is performed an educational campaign will be carried out to change the misconception and reduce the human bats conflict at the focal point where the conflict happens. The goal of campaigning is to increase awareness of the importance of a healthy and undisturbed relationship between humans and the animal kingdom with special emphasis on the dangers that unregulated contact between humans and wildlife will only increase the likelihood of developing a similar situation as of COVID-19. Overlay the project aims to change the negative views that many people carry with themselves towards bats especially in this pandemic times and to strengthen conservation policies to protect this species in Asian countries.

## Summary of Project Details

| | |
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| **Total project cost:** | EUR 514,791.2 |
| **Project title:** | Conservation Efforts to Protect Bats from Societal Misconceptions |
| **Project start date:** | 07 of January 2020 |
| **Project end date:** | 30 of August 2023 |
| **Project country:** | Indonesia, India, Vietnam, and China |
| **Up to 6 key-words:** | conservation, bats, Asian, covid-19, conflict, perception |

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| **Project lay summary:** | COVID-19 might represent a risk for bat conservation in different Asian countries. This project will compare how negative views induced by society by fear towards COVID might have affected the relationship between both humans and bats through surveying. Additionally, great effort would be put into mapping and addressing focal points of conflict with tailored and informative campaigns that would make emphasis on the fact that further unnecessary intrusion into bats habitats will result in more dangerous situations. The aim of the project is to relieve and change people's perceptions towards this animals for the better as they are a very important organism for most ecosystem around the world. |

## Project description

**Core project description**

**a. Background**

As we are currently facing a biodiversity crisis, the importance of animal conservation could not be any more relevant in this time and era. Recently there have been many successful cases where animal populations are saved from the brink of extinction thanks to the efforts of environmental campaigns such as saving the polar bear.

Unfortunately, not all animals have the same charm and in the case of bats there are even negative perceptions made by societies associated with them (devilish night creatures, blood suckers among others). Bats together with mice and snakes were animals connected with the self-reported fear or phobia (Robin and Reiger, 1991; Hoffmaster et al., 2015). Mostly in society, all bats species are considered as blood-sucker, when fact there are only three species of bats suck blood and two of these species suck blood from birds, and the common vampire bats (*Desmosus rotundus*) feed on large livestock (Mayen, 2003) and pose no serious threats to humans and pets. Previous studies showed that the level of knowledge has significant influence on attitudes and belief in myths about bats (Prokop et al., 2015; Hoffmaster et al., 2015).

In Asian countries the bats population is threatened in many ways such as by direct human impacts via hunting, in addition to urbanization and changes in land use. The current pandemic situation is adding even more tension to the human-bat relationship with their origin allegedly being traced down to bat origins (Cerri et al, 2020; Zhao, 2020).

Bats have important roles in the ecosystems as primary, secondary, and tertiary consumers that support and sustain both natural and human dominated ecosystems ranging from the simple to the complex. It has rich dietary habits from species that feed on insects and other arthropods to those that feed on fruit, nectar, and flowers (Kunz et al., 2011). Therefore bats have a great ecological important role as seed dispersers, pollinators especially in Southeast Asia, the Durian tree are only pollinated by the Dawn bat, *Eonycteris spelaea* and also insect population controllers. Even though there are a plethora of organizations trying to preserve bat populations (Voigt and Kingston, 2016) little has been done to understand how

COVID-19 affected the perception of human societies towards bats (MacFarlane and Rocha, 2020). That is why this project will look to answer the following questions

**b. The question or hypothesis to be tested**

1. Is the current pandemic situation adding more conflict and misconceptions towards bats in Asian countries?
2. Countries which have been severely affected by COVID-19 are more likely to concur to a greater number of conflicts with bats
3. Environmental care from governments and proximity between human populations and bats are direct factors that would affect the number of conflicts between them
4. Increased conflicts between human and bats are mainly due to fear of COVID-19 and new tailored educational campaigns will reduce the confrontation between these two groups.

## c. An outline of the methods to be used

**Survey** (question and hypothesis 1 - 3) The study is based on primary data that will be collected from 4 different countries in Asia: India, Indonesia, Vietnam, and China. Every country which is selected has a different impact on COVID-19 (mortality and socio-economically) based on the data from www.worldometers.info/coronavirus/ and UNDP, 2020 and environmental performance using the Environmental Performance Index (EPI) developed by Yale University. In every country, the area of the survey will be divided into two, which in each area will have two representatives: the rural area near the habitat of bats and the urban area which is far from the bat's habitat.

The survey will be performed by face–to–face interviews with 1000 people in the chosen region. As we are doing research on rural areas where wifi connections might not be accessible for everybody we believe this would be the best approach. A well-structured but simple questionnaire will be designed to collect information from the respondents. The questionnaire will be designed to study the perception of respondents towards bats in comparison with other animals that potentially could also transmit the same family of viruses. Every questionnaire will be provided with five different animal pictures: bats, pangolins, civet, camel, and mink. Respondents would have to rank the given pictures from the most approachable animals and the least friendly (dangerous). After showing the pictures the interviewers will explain the roles of these animals in the spreading of zoonotic disease and also COVID-19, and ask the respondents to re-rank the pictures.

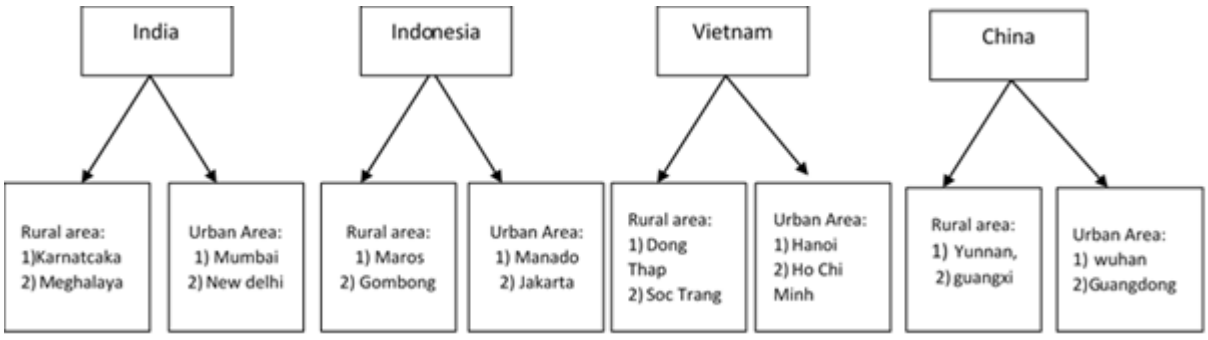


Fig 1. Survey location in 4 different countries

**Conflict mapping and campaign** (Hypothesis 4) the data used for mapping of human-bats conflict from every country (India, Indonesia, Vietnam, and China) will be collected from the authoritative media outlets from the internet. The analysis of conflict will be started by studying the temporal dynamics of words related to “bats”, “human-bats”, “killing the bats”, “covid” and the name of each country. Every human-bats conflict that happens from December 2019 to December 2020 will be recorded.

Information extracted from these reports included: village names, sub-district, district and province information, the status of conflict (i.e., if underway or finished/resolved), start and end dates of the conflict, and whether the conflict is still underway at the time of the report, conflict descriptions, impacted local communities, major ethnic groups, and livelihoods (Abram et al., 2017).

At the focal points where the highest number of conflicts are happening in each country (conflicts more than 20), a plan will be drawn to deliver tailored campaigns at educational centres. This campaign will be addressing the importance of bats in their ecosystems as well as the relationship between bats and COVID-19. The premise of the campaign is quite simple and straightforward, we depart from the idea that awareness and education will shift the negative perception most people in these locations might have due to the influence of COVID. Changes of perceptions towards animals thanks to conservation campaigns has been recorded before and an ideal example happened after the release of the movie “Jaws”, a single event affected negatively how people perceived sharks however with education and research efforts slowly white sharks’ populations are recovering from the hard blow made by the movie (Allan, 2020).

The campaign will be carried out for 6 months through social media platforms, radio, informational brochures/booklet and direct talk. After the campaign spread and distributed, the conflict mapping will be performed at the same places with the conflict report that occurred in the last months after the campaign took place. The measurement of conflict data will be carried out at the same amount of months as before.

**Statistica Analysis** (question and hypothesis 1-4) The data survey will be analyzed using nvivo 12-qualitative data analysis. The mapping of conflict will be extracted location information based on the named region(s) in every country. Locations will be georeferenced by searching for the region name (and any sub district or other administrative information) in gazetteers and online place name databases (Google Earth, Geographic Names http://www. geographic.org/geographic_names/and Wikimapia), and assigning the geographic coordinates if a confident match could be made.

## d. expected outputs

As mentioned previously our objective consists of outlining whether the current pandemic situation has done anything to shift the perceptions of humans towards bats in Asian countries. Once it is determined if there is any significant effect or not the project also aims to develop a campaign regardless of the results; such campaign will reinforce the fact that animals are not at blame for the current worldwide situation and incurring in dangerous hunts/conflicts will only increase the likelihood of exposing ourselves to more delicate situations. The campaign aims to increase awareness of the importance of a healthy and undisturbed

relationship between humans and the animal kingdom. Additionally, the project also hopes to change the way people think about bats or at least eliminate misconceptions unjustly given to them.

### e. expected timescales

| No | Research task | 2021 | | | | | | | | | | | | 2022 | | | | | | | | | | | | 2023 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | Survey Design, Running pilot for survey, traveling preparations, setting up all logistics with allied organizations. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | Supervisor allocation to each destination and travel (supervisor will carry out an supervise all operations and communicate with each other) Training coordinators and Initial phase of surveying | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | Survey in 4 different countries | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | Regroup of all supervisors of the surveyors to compile figures and data together as well to discuss findings. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | Parallely mapping of conflicts in all different countries from the previous year | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | Campaign design, Running pilot for the campaign, traveling preparations, setting up all logistics with allied organizations (Universities, NGOs). | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | starting campaign in different countries and regions* (*continuous task) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | Regroup of all the staff of the campaign to compile figures and data together as well to discuss findings. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 9 | Allowing some time for accurate data we proceed to do second mapping of conflicts in each countries to determine if | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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| | our campaigning effectively addressed the issue at hand | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | Parallely mapping of conflicts after campaign in all different countries. Conclusion | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Additional questions:**

**What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?**

With the current situation as it is our biggest concern would be to maintain the regulations and biosecurity measurements at all times, especially when surveying people on the streets. Additionally local travel insurance and health insurance are also a must for moving around in different countries

**Please provide details of agreed collaborations and project partners that will facilitate the proposed project:**

Partnership with different bat conservation organizations, universities from different countries will ease the acquisition of local help. Bat Conservation India Trust (India), Hanoi University of Natural Science (Vietnam), Indonesian Institutes of Sciences (Indonesia), Wuhan University (China)

**Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:**

A room with access to internet would suffice to perform any data analysis, as for the campaigns we are counting to bring with us our own projecting equipment therefore a large conference room with enough space to host people without compromising the biosecurity measurements

**Please provide details of necessary permits/licences obtained, if applicable:**

not applicable

**Please indicate how you will assess the scientific impact of the project and the benefits for non-academic audiences:**

Great part of the research done is with the hope of changing the negative view that most people have toward bats. A decrease in the bush bat meat and conflict would be a good indication that people realise the importance of such animals. Moreover, we hope that local governments strengthen their environmental laws towards bat protection as we could imagine that COVID is not improving their reputation by no means.

**How do you intend to make your research data publically available?**

Journals on animal conservation would be the most suited for a topic like ours. Additionally out ppt file from our campaigning will also be shared with our partners organizations to be publicly accessible in case someone wants to access it

**Please provide details of any published papers relevant to this project:**

Abram, N., Meijaard, E., Wilson, K., Davis, J., Wells, J., Ancrenaz, M., Budiharta, S., Durrant, A., Fakhruzzi, A., Runting, R., Gaveau, D. and Mengersen, K., 2017. Oil palm–community conflict mapping in Indonesia: A case for better community liaison in planning for development initiatives. Applied Geography, 78, pp.33-44.

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Cerri, J., Mori, E., Ancillotto, L., Russo, D. and Bertolino, S. (2020), "COVID-19 has led to a global increase in web searches for bats: a risk for conservation ?", EcoEvoRxiv, 29 April, available at:https://doi.org/10.32942/osf.io/4pa2q.

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Mayen, F. (2003). Haematophagous bats in Brazil, their role in rabies transmission, impact on public health, livestock industry and alternatives to an indiscriminate reduction of bat population. *Journal of Veterinary Medicine, Series B*, *50*(10), 469-472.

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## Budget

Equipment

| Item | Quantity | Description | Total Cost (€) |
|---|---|---|---|
| **Survey** | | | |
| **1.** Data Recorder Packs | USB drive : 60 x 4 countries<br>Hard disk: 2 x 4 countries | Data Recorder Packs to record the collected data | 607.1 |
| 2. Laptop | 2 x 4 countries | To store information, data, and multimedia content, upload and download information from the web. | 4,619.5 |
| 3. Printer | 2 x 4 countries | To print documents (letters, contracts, certificates, etc.) | 660 |
| 4. Camera | 2 x 4 countries | To take pictures. | 460 |
| 5. Software: Nvivo12 | | Data analysis software | 1,030 |
| 6. Safety Equipment (First aids, face shields, and masks) | 61 x 4 countries | For the protection of life and to avoid injuries or casualties. | 3,018 |

| | | | |
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| **Campaign** | | | |
| 1. Projector | 1 x 4 countries | to show the information (text, images, videos, etc.) large enough for people to see. | 660 |
| 2. Sound system (rent) | 1 x 4 countries x 24 campaign for 6 months | Medium Event System (2 speakers/stands, 2 wireless microphones, mixer) | 202 |
| 3. Laptop | 2 x 4 countries | To store information, data, and multimedia content, upload and download information from the web. | 4,619.5 |
| 4. Printer | 2 x 4 countries | To print documents (letters, contracts, certificates, etc.) | 660 |
| 5. Camera | 2 x 4 countries | To take pictures. | 460 |
| 6. Safety Equipment (First aids, face shields, and masks) | 17 x 4 countries | For the protection of life and to avoid injuries or casualties. | 1,345.6 |
| Overall Equipment Cost: | | | 18,341.7 |

Consumables

| Item | Quantity | Description | Total Cost (€) |
|---|---|---|---|
| Survey | | | |
| 1. Office supplies | 2 bundles office kits x 4 countries | Essential items including writing pads, pens, tape with dispenser, paper clips and more | 461.7 |
| 2. Communications | 61 x 4 countries x 1 month | Phone credit and Internet bills | 14,083 |
| 3. Questionnaire forms | 6 sheets x 1000 respondents | 5 sheets of A4 sized pictures of animals and 1 sheet of paper to rank the animals. | 700 |
| 4. Respondent | 1000 merchandises | Bats booklets and | 10,000 |

| | | | |
|---|---|---|---|
| compensation | | office kits. | |
| **Campaign** | | | |
| 1. Office supplies | 2 bundles office kits x 4 countries | Essential items including writing pads, pens, tape with dispenser, paper clips, and more | 461.7 |
| 2. Communications | 2 speakers + 15 staffs x 4 countries x 6 months | Phone credit and Internet bills | 23,550 |
| 3. Poster and Banner | 1 poster x 4 countries<br>1 big banner x 4 countries<br>2 stand banner x 4 countries | Creative and interactive posters and banners to draw the attention of the people and tempt to learn more about bats. | 300 |
| 4. Creative Booklets | 25 booklets x 4 countries x 24 campaign for 6 months | The booklets contain information about the campaign | 19,781 |
| 5. Bat merchandises | 25 bat merchandises x 4 countries x 24 campaign for 6 months | The merchandises to help promote the campaign program | 30,000 |
| Overall Consumables Cost: | | | 99,337.4 |

Personal and Field Travel/Accommodation/Subsistence

| Description | Total Cost (€) |
|---|---|
| 1. International travel to 4 countries | 4000 |
| 2. Domestic travel in each countries for one month survey (Rental costs, fuel costs, insurance, repair costs)<br>a. Indonesia<br>b. India<br>c. Vietnam<br>d. China | 5,000<br>7,775.3<br>4,613.7<br>10,328 |
| 3. Accommodations for surveyor for one month (rent house/apartment)<br>a. Indonesia<br>b. India<br>c. Vietnam<br>d. China | 8590.3<br>10,230.6<br>10,938.3<br>15,411.6 |

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| 4. Subsistence for surveyor for one month (Foods and drinks)<br>a. Indonesia<br>b. India<br>c. Vietnam<br>d. China | 4,276<br>3,751<br>4,135.9<br>7,703.6 |
| 5. Domestic travel in each country for 6 months campaign (Rental costs, fuel costs, driver salaries, insurance, repair costs)<br>a. Indonesia<br>b. India<br>c. Vietnam<br>d. China | 1,857.7<br>3,045.6<br>2,008.5<br>3,031.8 |
| 6. Subsistence for campaign staff for 6 months<br>a. Indonesia<br>b. India<br>c. Vietnam<br>d. China | 1,521.8<br>1,247.7<br>1,330<br>2,878.4 |
| Overall Travel Cost: | 109,676 |

Employment (note only casual, short term assistance will be considered)

| Position | Description of role | Rate & Duration of employment | Total Cost (€) |
|---|---|---|---|
| **Survey** | | | |
| Supervisor | The survey supervisor will direct, plan, coordinate, and oversee the surveyors. A total of 4 surveyors in each country will be selected. | a. Indonesia: 584/month<br>b. India: 390.8/month<br>c. Vietnam: 600/month<br>d. China: 883.8/month<br>Duration of employment: 1 month | 4,672<br>3,126.4<br>4,800<br>7,070.4 |
| Surveyor (Students) | Surveyors that consist of 60 each country students will interview 1000 respondents for a month. Each surveyor to obt ain information by asking question from a prepared questionnaire. Tallies and/or information collected. Enters collected data into computer. | a. Indonesia 1.05/hour<br>b. b. India 1.17/hour<br>c. Vietnam 0.95/hour<br>d. China 1.26/hour<br>Duration of employment 160 | 40,382<br>45,052.5<br>37,864.5 |

| | | | |
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| | Make a documentation (pictures/video) from the survey and to be responsible with the supervisor. | hours. | 48,498.2 |
| **Campaign** | | | |
| Speakers (Academic) | Speakers of the campaign responsible for writing and outlining speech scripts, and delivering high-quality speeches to audiences, also be required to answer audience questions and interact with members of the public. | 150/campaign<br><br>Rate & Duration of employment : 24 campaign | 28,800 |
| Staffs (Students) | Staffs of the campaign will promote the campaign by sending out fliers, advertising on TV, radio, and the Internet, and arranging for campaign, they help to give information about the campaign to the public. | a. Indonesia 1.05/hour<br>b. India 1.17/hour<br>c. Vietnam 0.95/hour<br>d. China 1.26/hour<br><br>Duration of employment 192 hours. | 12,102.6<br><br>13,509.6<br>11,357.7<br><br>14,543 |
| Multimedia producer | A multimedia producer oversees digital productions of visual documentation for both videos and pictures including creative direction, filming, and post-production editing while providing technical and creative direction and support. | 247.5/campaign<br><br>Duration of employment 24 campaign | 23,760 |
| Overall Employment Cost: | | | 254,636 |

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| Total project cost | **€ 514,791.2** |

# 3. Understanding supermarket customers behavior towards the consumption of single-use plastics during the COVID-19 pandemic as a possible environmental threat

## Applicants names

Miao Dang, Jiahui Zhang, Luis Eyzaguirre

## Abstract

Over time, plastic production gives human beings a wide range of solutions, making our life easier, both for the industrial sector and for everyday use, making their demand higher. However, the consumption of single-use plastics (SUP) during this last decade increased, where one of the most relevant suppliers are supermarkets, making it a global problem due to their small life cycle and their high persistence in the environment. The pollution of single-use plastics (SUP) not only harms the ecological environment but also poses a threat to the health of animals, plants and even humans. Last year the outbreak of the novel COVID-19 hit the globe, producing a high sanitary emergency, forcing supermarkets to increase their sanitary and hygienic measures. These measures are traduced in the usage of masks, gloves and full plastic-wrapped products. Anyhow, one of the most important factors of the high SUP demand is customers. The theoretical approach in relation to sustainable environmental care is related to anthropogenic behavior, in this way, this research study tries to relate the behavior and the perception of the consumer in relation to environmental care. Previous studies have shown that the behavior and perception of customers, which are one of the most relevant stakeholders, are the key to understanding the high demand for certain products, in this case SUP. Situated in the above mentioned, we aimed to understand the perception of supermarket customers towards SUP during the COVID-19 pandemic. Our first hypothesis states that the COVID-19 outbreak produced a change in the supermarkets customers perception towards SUP, increasing its consumption. To evaluate our prediction, we decided to perform surveys based on the Likert scale method among supermarket customers through Social networks and in person surveys in 2 capital cities, Warsaw, Poland and Lima, Peru, with distinctive cultures and SUP regulations. In this way, apart from analyzing consumer behavior, it is also possible to evaluate whether customers think it is more important to raise awareness of COVID-19 prevention and control than SUP pollution. And assume that these customers are now more health-conscious, which means more SUP consumption.This study was designed basically to have a reliable database that shows us the behaviors and perceptions of customers towards SUP consumption in 2 different countries. Due to this, in long terms it will be possible to propose social solutions in front of the unsustainable high demand of SUP around the world.

## Summary of Project Details

| | |
|---|---|
| **Total project cost:** | 113 079 USD |
| **Project title:** | Supermarket customers behavior towards single-use plastics consumption during the COVID-19 pandemic |
| **Project start date:** | 01/03/2021 |
| **Project end date:** | 30/09/2022 |
| **Project country:** | Warsaw, Poland and Lima, Peru |
| **Up to 6 key-words:** | Single-use plastics, Sustainability, COVID-19 pandemic, Behavior, Perception |


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| **Project lay summary:** | Due to the high threat that represents the uncontrolled single use plastic (SUP) demand and acquisition, it is important to evaluate the behavior and perception of the main stakeholders in the SUP industry, in this case supermarket customers. The main aim of this project is to understand the perception of the customers towards SUP during the COVID-19 pandemic. This study will be managed by surveys through social media and personal surveys, using questions in a scale method (Likert test), in which we are going to evaluate the perception of the customers and how it changes due to the global sanitary crisis. In this way, we are going to be able to create a reliable database, in which we can conduct future programs and suggest social solutions to SUP high demand . |


## Project description

**Core project description**

### 1. Background

Over time Plastics products provide enormous social benefits to human beings. While convenient, single-use plastic products also put a heavy strain on the environment, due to their small life usage cycle and their extremely high persistence in the environment. Throwaway culture has turned plastic products into garbage after a single use, which is convenient for the people and society due to their simplicity, but it is also highly harmful for the environment and in long terms for the human being[1][3]. Owing to the above mentioned

environmental and health concerns associated with plastic pollution are a long recognised as a global problem. Whilst approximately 10% of all solid waste is plastic, up to 80% of the waste that accumulates on land, shorelines, the ocean surface, or seabed is plastic[2]. The mismanagements, also with the high production capacity of plastic products, on the plastic solid waste, raise a prosperity study validates that plastics has universally permeated to the aquatic ecosystem nowadays. Eighty percent of the yearly 8 million tons of plastic that enter the ocean is single use plastic, such as plastic bottles, plastic shopping bags[3].

The outbreak of COVID-19 has affected people's lives to some extent. Staying at home, limiting travel and isolation are the most effective preventive measures. But the government fears that the economy will collapse and a crisis will occur. Governments have therefore made different choices. COVID-19 is not only a direct threat to human life and health, it has also triggered another outbreak. Disposable plastics are making a worldwide resurgence. Surgical face masks are widely used as protective tools. According to WHO estimates, approximately 89 million masks are needed to control COVID-19 each month[4]. This has led to an astonishing increase in the production of surgical face masks worldwide.

Due to the mentioned above, it should be noted that the demand for SUP, single-use plastics, was increased before the pandemic. However, as previously mentioned, SUP products are being used overwhelmingly, due to the sanitary regulations imposed by competent organizations [5]. However, previous studies have concluded that one of the most important suppliers of single-use plastics are supermarkets due to their simplicity and low cost, it is still a huge challenge to reduce their use. Due to all this we are targeting our study to one of the main stakeholders in supermarkets, which are the first common consumers of SUP products[6].

Previous studies carried out in the United Kingdom, made clear that the analysis of customer perception and behavior is one of the most important points to understand the demand of a product, in this case SUP [7]. Due to the pandemic, we know that there are disruptions in the behavior and perception of supermarket customers towards SUP products. However, there is no reliable database that tells us how people react to this health hazard. Our study analyzes the behavior based on the customer's perception of SUPs in supermarkets. In this way, we are going to be able to have a reliable database which will allow us to offer future solutions [7][8].

To sum up, from the study of human behavior, it can be seen that motivation is the process of causing behavioral choice, and the degree of motivation is different. If it needs to predict people's behavior, it can by observing the attitude. Motivation determines its attitude. Specific behavior alone is not enough to predict the motivation of the target. In this research, consumers are in a complex environment, and external factors such as the environment (pandemic), government policies, and internal factors (worries about health) will all become motivations that affect people's attitudes. Therefore, during the COVID-19 pandemic, consumers' consumption habits for SUP are unpredictable. In order to understand the changes in supermarket customers as consumers' habits of using SUP plastics from the perspective of personal behavior and perception during the COVID-19 pandemic. In addition, Warsaw and Lima belong to countries that have different policies and cultures for SUP, so choosing them as the research site can better conduct comparative studies and

understand the response of people in different regions to health hazards from an objective perspective. In this way we can avoid falling into bias and have a more concrete and robust database.[9]

## 2. The question to be tested.

Main question:

1) Does the COVID-19 pandemic modify Supermarket Customers perspective towards the consumption of SUP?

Specific questions:

1) How different is the perception of supermarkets customers towards SUP between Warsaw and Lima during the pandemic?
2) Does health concern displace the environmental concern in supermarkets customers during the pandemic?

## 3. Outline of the methods to be used.

In this study we are going to analice 2 countries, essentially in the capitals cities of each county, Lima, and Warsaw. Moreover, the methodology applied will be based on the application of a Likert scale survey, performed by online surveys and in person interviews. Overall, this project will be carried out in 4 steps, which will be described below. Before all, Sustainability in terms of environmental care also includes the study of sustainable consumption in relation to the social structure where the study is carried out, where the consumer and the sociology of consumption play an important role in providing environmental solutions that are generated by unsustainable consumption. Additionally, understanding consumer behavior in the face of a world-scale shock will provide a critical framework for research on sustainability regarding the use of single-use plastics [10].

The social approach and sustainable consumption are totally relevant for this research study, since the consumption of single-use plastic is currently considered an unsustainable practice that causes damage to the biophysical, social and even economic environment, as already stated. In this study, the usage and relatively short life of single-use plastic products is questioned and others relate this to the health issues that are being taken nowadays. In order to address the issue related with environmental concern and health in relation to the use of single-use plastic in supermarkets, the methodology focuses on conducting a questionnaire using the likert scale as a technique [10]. We will use the Likert scale method for the surveys, since respondents specify their level of agreement or disagreement on a symmetric scale for a series of statements. Therefore, this rank captures the intensity of their feelings and perceptions for a certain topic. Due to its peculiarity, the methodology of surveys using Likert scales has been widely used in the fields of psychology, social sciences, statistics, business and marketing[10]. In this way, we will be able to analyze and understand in a precise way the perception and behavior of consumers in relation to the current pandemic. The questionnaire consists of 2 parts. The first part, we will address aspects focused on consumer perceptions prior to the pandemic towards single use plastics. The second part will focus on key perspectives during the pandemic towards single use

plastics. It should be noted that both parts are included in a single survey, previously conducted studies applied this sensing method to analyze the change of perception in individuals in relation to the objective studied, and it seems to be a efficient way to evaluate these changes over time considering the obstacles presented by the actual pandemic situation[11]. In each part, we will seek to know the consumption habits of the clients, the environmental awareness that they have and find what factor produces this change in their perception. As we can see in figure 1, we want to know how the perception of the consumers change towards single use plastics, in order to understand their behavior in front of the pandemic and explain their consumption. In the other hand, we decide to undergo these surveys in the capital cities of each county, basically because the amount of people is higher and also because of the broad variety of supermarkets they have, this is a good indicator for us to evaluate the behavior of customers in front of a high variety of supermarkets in a large population, which will give us a more significant value to our statistical analysis. In addition, the COVID-19 pandemic hit in high measure in both capital cities[12], making people more aware of the pandemic and their sanitary measures[12].

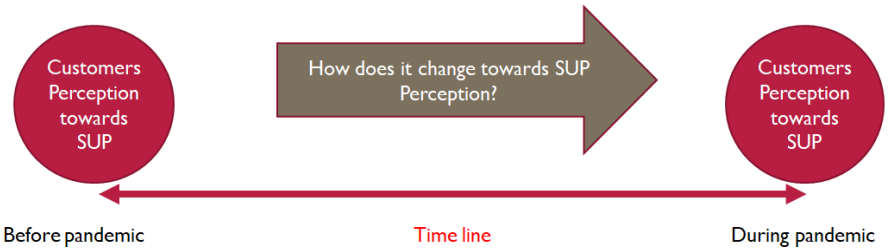


Figure 1. Outline of the study design. Based in the survey, it is sought to know the change of the perception of supermarket customers toward single use plastics. (source: Own production)

The steps that we are going to follow, for this study are:

1) Build the survey for each country in a standard format to be applicable in both countries. For each country we are going to perform 4000 surveys with a prediction of a 10% of responses. Considering our Sample size with a 95% confidence level it is going to be 400 responses, this calculation was done by the statistical program: Stata 16 free trial. Each survey will address the perception of the consumers prio pandemic and during pandemic in a likert scale.
2) The first group of surveys will be carried out through social networks, in which we are considering a broad variety of options: Instagram, Facebook, Twitter, Whatsapp, Tumblr, Reddit, etc. In this way we are going to be able to reach a higher amount of people and answers. In this first stage we are considering 2000 surveys in which we expect 200 responses per country according to our previous calculation. For this stage, we are going to hire 5 marketing and social media analysts per country, to run out the surveys in specific social networks groups compelled for each district, in each capital city.

3) The second group of surveys will be carried out by personal surveys. In this step we are considering 2000 surveys in which we expect 200 responses per country according to our previous calculation. In this step we need to hire 20 pollsters. On the other hand in Warsaw we need to hire another 20 pollsters.
4) The last step is basically to perform our database and start the statistical analysis in which we are pretending to use stata 16: Data analysis program us our main analysis program.

## 4. Expected outputs.

Main Hypothesis:

1) The COVID-19 outbreak modified the perspective of customers which altered their behavior towards SUP, increasing their demand in Lima and Warsaw.

Specific Hypothesis:

1) The perception towards SUP will be different in both capitals cities, due to the plastic regulations and social perspectives, Lima will have a higher demand of SUP than Warsaw during the pandemic.
2) The environmental concerned customers turned to health concerns in relation with SUP consumption during the pandemic.

## 5. Expected timescales.

| Start Date | End Date | |
|---|---|---|
| 01/03/2021 | 31/03/2021 | Surveys Preparation |
| 01/04/2021 | 30/04/2021 | Run out Pilot Surveys |
| 01/05/2021 | 31/05/2021 | Corrections and feedback from the survey |
| 01/06/2021 | 31/10/2021 | Run surveys (Social network surveys and Personal surveys) |
| 01/11/2021 | 31/12/2021 | Perform data base |
| 01/01/2022 | 31/03/2022 | Statistics |
| 01/04/2022 | 30/06/2022 | Draft writing |

| | | |
|---|---|---|
| 01/07/2022 | 30/09/2022 | Corrections Publication |

**Additional questions:**

**What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?**

One of the potential risks we are facing is that our they can be infected with COVID-19 during the personal survey, however, one of the measures we are taking in front of this threat is running out the other half of surveys via social networks, in this what we are reducing significantly the over exposure of our employees. On the other hand, we also encourage them to use sanitary measures by wearing surgical face masks and gloves in work time and do hand disinfection every 30 minutes.

**Please provide details of agreed collaborations and project partners that will facilitate the proposed project:**

We as students of the Jagiellonian University and member of team research of the institution, have the collaboration with the competent organization to carry out the project. The institutions will also appear in our publication as the main institutional partner.

**Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:**

The project will be carried out at Jagiellonian University and required for applicants research thesis in the Master's programme. The laboratories, computers need to be required and software Stata 6 will be used for the data analysis process.

**Please provide details of necessary permits/licences obtained, if applicable:**

The surveys that will be carried out for this project are going to be completely anonymous according to the Official Statistics Act of 29th June, 1995 (Journ. of Law 2016, position 1068, with amendments), Statistics Poland guarantees the security of the personal information of the individual. On the Other hand, these surveys are going to be intended for people older than 18 years old, due to the protection of the under age individuals.

**Please indicate how you will assess the scientific impact of the project and the benefits for non-academic audiences:**

By investigating supermarket customers' behaviours in the consumption of single-use plastics in Warsaw and Lima during the COVID-19 pandemic, its results can provide a data basis for the formulation of plastic-related social solutions, also it can be used for the competent organization to formulate laws and regulations. At the same time, it provides new ideas for publicizing environmental protection and treating single-use plastic pollution after the pandemic.

**How do you intend to make your research data publically available?**

The availability of the database and the research paper will be provided by the direct contact with the authors via researchgate or Email. The information will be available for academical and strictly for social and environmental purposes.

**Please provide details of any published papers relevant to this project:**

[1] Graca, B., Szewc, K., Zakrzewska, D., Dołęga, A., & Szczerbowska-Boruchowska, M. (2017). Sources and fate of microplastics in marine and beach sediments of the Southern Baltic Sea—a preliminary study. Environmental Science and Pollution Research, 24(8), 7650-7661.

[2] Wabnitz, Colette & Nichols, Wallace. (2010). Editorial: Plastic Pollution: An Ocean Emergency. Marine Turtle NewsLetter. 20.

[3] McDermott, Kristin L. (2016). Plastic Pollution and the Global Throwaway Culture: Environmental Injustices of Single-use Plastic. ENV 434 Environmental Justice. 7.

[4] WHO. (2020). Shortage of Personal Protective Equipment Endangering Health Workers

Worldwide. Who.

[5] Wagner, T. P. (2017). Reducing single-use plastic shopping bags in the USA. Waste Management, 70, 3-12.

[6] McNicholas, G., & Cotton, M. (2019). Stakeholder perceptions of marine plastic waste management in the United Kingdom. Ecological Economics, 163, 77-87.

[7] Vince, J., & Hardesty, B. D. (2018). Governance solutions to the tragedy of the commons that marine plastics have become. Frontiers in Marine Science, 5, 214.

[8] Urban-Malinga, B; Zalewski, M; Jakubowska, A; Wodzinowski, T; Malinga, M; Palys, B; Dabrowska, A. (2020). Microplastics on sandy beaches of the southern Baltic Sea. Marine Pollution Bulletin, 155, 111170.

[9] Wang Yanzhi. (2016), Research on the Driving Factors of Customer Customization, Beijing Book Co. Inc.,6, 226.

[10] Bennett, D. E. (2013). Geography and the emergence of sustainability science: missed opportunities and enduring possibilities. The Geographical Bulletin, 54(2), 99.

[11] Cimerman, S., Chebabo, A., da Cunha, C. A., & Rodríguez-Morales, A. J. (2020). Deep impact of COVID-19 in the healthcare of Latin America: the case of Brazil. Braz J Infect Dis.

[12] Smith-Sebasto, N. J., & D'Costa, A. (1995). Designing a Likert-type scale to predict environmentally responsible behavior in undergraduate students: A multistep process. The Journal of Environmental Education, 27(1), 14-20.

## Budget

Equipment

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| Stata 16 | 1 | Software for Statistics and Data Science | 225 USD |
| Overall Equipment Cost: | | | 225 USD |

Consumables

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| Printing | 10 000 | Standard quality print surveys | 410 USD |
| Pencils/Pens | 500 | 0.5 USD per each | 250 USD |
| Notebook | 500 | 1.3 USD per each | 650 USD |
| Post | 750 | 4 USD per kg, Poczta Polska | 24 USD |
| Overall Consumables Cost: | | | 1 334 USD |

Personal and Field Travel/Accommodation/Subsistence

| Description | Total Cost |
|---|---|
| PublicTransportation each person 125 USD per 3 months plus 1 month ticket for 48 USD in Warsaw, and 50 USD meals per month. Total amount calculated for 20 pollsters hired. Total amount for 4 months. | 7 460 USD |
| PublicTransportation each person 173 USD per 4 month in Peru. and 50 USD for meals per month. Total amount calculated for 20 pollsters hired. 4 months. Total amount for 4 months. | 7 460 USD |
| 1 plane ticket in KLM two ways Poland Perú | 1200 USD |

| | |
|---|---|
| Overall Travel Cost: | 16 120 USD |

Employment (note only casual, short term assistance will be considered)

| Position | Description of role | Rate & Duration of employment | Total Cost |
|---|---|---|---|
| Warsaw- part time job | Social network surveys: 5 employees<br>● The salaries includes insurance and taxes<br>● Gross amount: 600 USD | Four months | 12 000 USD |
| Warsaw- part time job | Personal surveys:20 employees<br>● The salaries includes insurance and taxes<br>● Gross amount: 600 USD per month | Four months | 48 000 USD |
| Lima - part time job | Social network surveys: 5 employees<br>● The salaries includes insurance and taxes<br>● Gross amount: 354 USD per month | Four months | 7 080 USD |
| Lima - part time job | Personal surveys: 20 employees<br>● The salaries includes insurance and taxes<br>● Gross amount: 354 USD per month | Four months | 28 320 USD |
| Overall Employment Cost: | | | 95 400 USD |

| | |
|---|---|
| Total project cost | 113 079 USD |

# 4. Effects of Rat Maternal Behaviour on Life History Outcomes and Reproductive Success in Male Offspring

## Applicants names

Alina Bondur, Mateusz Chechetkin

## Abstract

Individual's life outcomes are determined by a complex system of different interconnected factors and experiences. There is evidence suggesting that parenting behavior in species with parental care have prolonged effect on their development and later life outcomes. A traumatic childhood experience can result in permanent effects, including on the level of gene regulation (so-called epigenetic changes). These effects prepare individuals for surviving adversity, but can also make them anxious or lead to dysfunctions in behavior, including social behavior, with is especially relevant for social species and crucial for their survival and reproduction. This research aims to study the effects of early childhood stress on gene regulation and reproductive behaviour of a model social mammal, laboratory rat. The results are important both for fundamental science and, potentially, for improving our understanding of the effects of childhood traumatic experiences in social mammals in general, including humans.

## Summary of Project Details

| | |
|---|---|
| **Total project cost:** | 177 800 euro |
| **Project title:** | Effects of Rat Maternal Behaviour on Life History Outcomes and Reproductive Success in Male Offspring |
| **Project start date:** | 01.01.2022 |
| **Project end date:** | 01.06.2024 |
| **Project country:** | Poland |
| **Up to 6 key-words:** | stress, epigenetics, reproductive behaviour, social mammals |

| | |
|---|---|
| **Project lay summary:** | A complex relationship exists between an individual's life experiences, genetic background, and how they behave later in life. We know that for species that take care of their offspring, early childhood is crucial for development. A traumatic childhood experience can result in permanent effects, including on the level of gene regulation (so-called epigenetic changes). These effects prepare individuals for surviving adversity, but can also make them anxious or lead to dysfunctions in behaviour. Behavioural problems are especially relevant for social species and have consequences for their survival and reproduction.<br>Our research aims to study the effects of early childhood stress on gene regulation and reproductive behaviour of a model social mammal, laboratory rat. The results are important both for fundamental science and, potentially, for improving our understanding of the effects of childhood traumatic experiences in social mammals in general, including humans. |

## Project description

**Core project description**

**Introduction**

Post-natal development and parental care has profound consequences for behaviour and life outcomes of social mammals which is why early childhood stress in social mammals has persistent, often epigenetically determined effects across the individual's entire life history. These effects can be behavioural (such as increased nervousness and risk aversion), neurological (changes in perception and reaction to sensory stimuli) or metabolic (such as an increased tendency to gain weight; Essex, 2013) .

In the case of epigenetically determined changes, the effects can be trans-generational and be passed on to the offspring. These mechanisms have evolved to protect the individual and help them overcome adversity and survive in a hostile environment, however, they can also have negative effects such as anxiety or, in social species, worsened social functioning (Avital, 2006) . Therefore, this stress response represents an evolutionary trade-off.

One part of this tradeoff is consequences for reproduction, especially when more complex reproductive behaviour is typical for the species. Early childhood stress has been linked to lowered reproductive success in social mammals (Pohl, 2007) . As appropriate behaviour during courtship is required for successful mating, any factors disrupting social behaviour are expected to have an impact on reproductive success in social mammals (Jablonka, 2009) . This stress can be induced by inappropriate maternal behaviour, e.g. in

rats so-called “bad mothers” have been identified based on lack of appropriate parental care such as grooming (Oomen, 2011) .

Many studies have addressed the effects of this type of stress on early life outcomes and survival (Zucchi, 2013; Shalev, 2016), but not much is known about the continued effects later in life, including on reproductive outcomes. Based on previous research, there are reasons to believe that:

- inappropriate parenting behaviour will incur permanent effects on the offspring
- changes induced by early childhood stress are epigenetic in nature and are independent of genetic background
- impaired social functioning will lead to lower reproductive success in males

Additionally, it is yet unknown whether:

- such epigenetic changes persist for an individual’s entire life history
- epigenetic markers of early childhood stress are passed on through sperm cells from father to offspring.

Our research aims to address these knowledge gaps by conducting several related experiments, assessing both molecular and behavioural effects of early stress on social mammals using the model organism of common rat *Rattus rattus*.

**Research hypothesis**

**Hypothesis 1**: Epigenetic markers of early childhood stress persist for an individual’s entire life history.

We speculate that the stress markers will persist well into adulthood and till death since there is evidence that behavioural changes associated with early childhood stress affect individuals for their entire lifespan and we hypothesize that these changes are caused by the epigenetic markers.

**Hypothesis 2**: Presence of early childhood stress correlates with lower reproductive success (measured as numbers of successful copulations).

We think that the adverse childhood experience in individuals will correlate with lower reproductive success because previous research shows correlation both between:

a) early childhood stress and maladaptive social behaviour in social mammals

b) appropriate social behaviour and higher reproductive success in males

**Hypothesis 3**: Epigenetic markers of early childhood stress are passed through sperm from father to offspring.

Previous research shows that epigenetic markers in mammals can be passed on from mother to offspring through both gametes and through various physiological effects during gestation; we speculate that a similar process leads to epigenetic markers being maintained in sperm of mammalian males and passed on to their offspring.

**Experiments and methods**

**Experiment 1**: Male rat pups of mothers with no history of inappropriate parenting behaviour are split into groups (one pup from the same litter goes to control group, another pup from that litter goes to the experimental group to exclude/minimize any genetic effects). 30 individuals are randomly split between and reared by 6 “good” foster mothers. 30 are randomly split between and reared by 6 “bad” foster mothers. Epigenetic stress markers in both groups are assessed at several point in life (during childhood, at sexual maturity, at an older age) and compared.

For sampling, brain tissue biopsies can be obtained using fine needle aspirate (FNA), which is a procedure that can be conducted multiple times on a living individual with minimal to mild impact on an individual’s functioning. For DNA methylation analysis, pre-made kit are available. Specific loci that will be checked for presence of methylation will be determined using existing literature.

**Experiment 2**: Reproductive success of individuals defined as number of successful copulations with a female is assessed through observation on video records and measured. There are developed protocols for maintaining video records as well as analysing the footage using a combination of automatic processing and personal observations. The values are then compared between various groups of individuals based on their developmental/life history.

In order to exclude the influence of medical intervention (biopsy) and stress associated with it, additional control groups will be used for individuals in both experimental groups. In total, two factors and four groups will be used in the behavioural experiments, and the results will be analysed using two-factor statistical analysis. A further explanation of factors and groups is represented in figure 1.

**Experiment 3:** Presence of epigenetic markers is compared between mature individuals, their gametes, and the offspring they produce. Epigenetic markers in brain tissue are assessed as detailed in experiment one. Same DNA regions are analysed in gametes following in vitro capacitation.

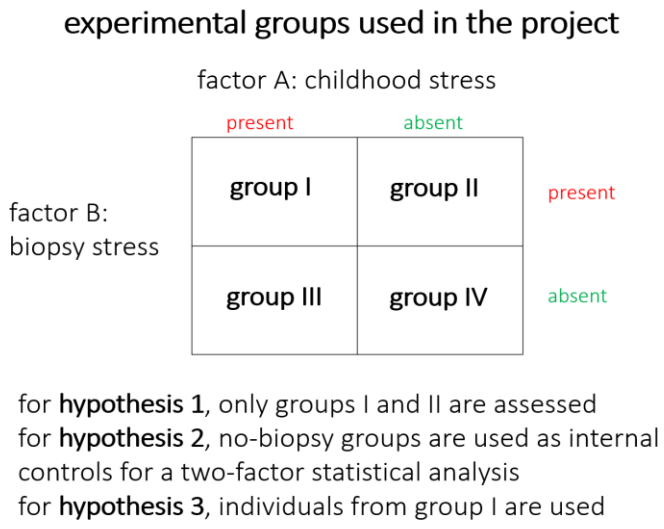


**Figure 1**: visual representation of factors analysed and groups used to test each hypothesis

**Expected outputs of the research**

**experiment 1**: results of DNA methylation analysis conducted on brain tissue samples of 60 individuals 3 times across their life history

**experiment 2**: reports of behavioural analysis of 120 individuals based on video records

**experiment 3**: results of DNA methylation analysis conducted on brain tissue samples of 60 individuals from the offspring of male rats from both control and experimental groups

On the basis of each experiment a scientific article will be written.

Expected timescale of the project is 30 months.

**Additional questions:**

**What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?**

The risks to health and safety are minimal as work will be conducted on standard laboratory animals indoors and tissue sample analysis does not require working with dangerous substances. All risks are minimized through appropriate health&safety education and maintenance of safety protocols during every stage of research.

**Please provide details of agreed collaborations and project partners that will facilitate the proposed project:**

All research will be conducted in the facilities of Jagiellonian University, specifically in the Institutes of Zoology and Institute of Environmental Sciences.

**Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:**

Jagiellonian University Institute of Zoology and Biomedical Research and Jagiellonian University Institute of Environmental Sciences will provide laboratory space and all necessary equipment. Funding is required for consumables and costs of maintaining a rat colony.

**Please provide details of necessary permits/licences obtained, if applicable:**

The permission of the ethical committee is required to conduct this research as invasive procedures (brain biopsy) have to be implemented for some of the animals used in the experiments.

**Please indicate how you will assess the scientific impact of the project and the benefits for non-academic audiences:**

The scientific impact will be assessed based on articles published in peer-reviewed literature as well as participation in at least one relevant conference (which will be privately funded). Research has potential for practical applications, e.g. for improving our understanding of childhood trauma and its impacts on individuals, which will benefit medical/psychiatric research and has a potential to improve lives of those living with trauma.

**How do you intend to make your research data publically available?**

Project budget includes sums necessary to ensure that the published research will be open access and freely available to all who wish to read it.

**Please provide details of any published papers relevant to this project:**


Avital, Avi, et al. "Effects of early-life stress on behavior and neurosteroid levels in the rat hypothalamus and entorhinal cortex." Brain research bulletin 68.6 (2006): 419-424.

Essex, Marilyn J., et al. "Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence." Child development 84.1 (2013): 58-75.

Jablonka, Eva, and Gal Raz. "Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution." The Quarterly review of biology 84.2 (2009): 131-176.

Oomen, Charlotte A., et al. "Early maternal deprivation affects dentate gyrus structure and emotional learning in adult female rats." Psychopharmacology 214.1 (2011): 249-260.

Pohl, Joanna, et al. "Repeated exposure to stress across the childhood-adolescent period alters rats' anxiety-and depression-like behaviors in adulthood: The importance of stressor type and gender." Behavioral neuroscience 121.3 (2007): 462.

Shalev, Idan, and Jay Belsky. "Early-life stress and reproductive cost: A two-hit developmental model of accelerated aging?." Medical Hypotheses 90 (2016): 41-47.

Zucchi, Fabiola CR, et al. "Maternal stress induces epigenetic signatures of psychiatric and neurological diseases in the offspring." PloS one 8.2 (2013): e56967.


## Budget

Equipment

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| | | | |
| Overall Equipment Cost: | | | |

Consumables

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| DNA methylation analysis kits | 10 | Full set of components needed to detect DNA methylation | 2000 euro |

| | | | |
|---|---|---|---|
| Materials for sustaining rat colony | 1200 | Food & water portions per day per colony and bedding per cage | 5000 euro |
| Materials for extracting and processing nervous tissue | 30 | Extraction kits + small additional equipment | 11 000 euro |
| Other laboratory consumables | | Paper, clothes, etc. | 1800 euro |
| Fees for open access publication | 3 | Cost of making a scientific publication open to all | 3000 euro |
| Overall Consumables Cost: | | | 22 800 euro |

Personal and Field Travel/Accommodation/Subsistence

| Description | Total Cost |
|---|---|
| | |
| Overall Travel Cost: | |

Employment (note only casual, short term assistance will be considered)

| Position | Description of role | Rate & Duration of employment | Total Cost |
|---|---|---|---|
| Researcher | Animal care, conducting experiments | 30 months * 2000 euro | 60 000 euro |
| Researcher | Animal care, conducting experiments | 30 months * 2000 euro | 60 000 euro |
| Technical worker | animal care | 30 months * 1200 euro | 36 000 euro |
| Overall Employment Cost: | | | 156 000 euro |

| | |
|---|---|
| Total project cost | 178 800 euro |

# 5. Moult costs and feathers' quality in young and old zebra finches

## Applicants names

Magdalena Honkowicz, Agata Różańska, Karolina Sorys, Małgorzata Śliż

## Abstract

Feathers are epidermal growths of birds. They have a complex structure and fulfil many functions. The most important are: barrier against adverse environmental conditions, insulation of the body, flight, display, and camouflage. Feathers, to perform these functions, are changed in the process called moult, since during use they lose their properties. The moult is costly for birds because they have to synthesize protein to produce new feathers. Also, they use much energy to regulate body temperature while feathers insulation is reduced and overcome less flight efficiency. Due to aging, the production of energy and the synthesis of protein is lowered. Thus, for older birds, fulfilling these requirements may be harder than for the young ones. However, there is nothing known about how aging affects moult costs, feathers' growth rate, and their characteristics. Since the feathers are crucial for birds' survival, such information will contribute to our knowledge of ecology, aging, and energy budgets of birds. Our study aims to examine possible changes in feathers' quality and moult costs in aging birds on the example of zebra finches (*Taeniopygia guttata*). The experiments will be carried out on young (one and two-year-old) and old (between five and six-year-old) birds. Using an induced moult, we will study the growth rate of the flight feathers. Also, we will examine differences in mechanics and structure of the flight feathers, including their mass, elasticity, and resistance to degradation by bacteria. Finally, we will examine how particular moult costs differ in young and old birds. We will simulate moult by trimming the feathers. With the use of climatic chambers, we will study how young and old birds deal with reduced insulation. A wind tunnel will enable us to study differences in flight efficiency among young and old birds.

## Summary of Project Details

| | |
|---|---|
| **Total project cost:** | 247 300 PLN |
| **Project title:** | Moult costs and feathers' quality in young and old zebra finches. |
| **Project start date:** | 01.01.2021 |
| **Project end date:** | 30.12.2021 |
| **Project country:** | Poland, Germany |
| **Up to 6 key-words:** | bird; moult; feathers; feather quality; aging; wind tunnel |

| | |
|---|---|
| **Project lay summary:** | Feathers are essential for the survival of birds. Feathers protect birds from cold, provide camouflage, and most importantly - enable flight. The feathers deteriorate over time, so birds replace old feathers with new ones in a process called moult. The production of new feathers is very costly. Besides, due to missing feathers, birds have to use extra energy to maintain body temperature and fly. Every organism gets old and, as a result, becomes weaker and less efficient. The costly moult may be a challenge for old birds. In this study, we want to check how aging affects moult costs, feathers' growth rate, and their quality. We will pluck selected feathers from birds' wings and check how fast they regrow. We will measure the feather's properties, like elasticity and resistance to degradation. Also, we will study how old and young birds deal with missing feathers in the case of flying and thermoregulation. |

## Project description

**Core project description**

### 1. Background and rationale

**Feathers** are epidermal growths of birds. They are built almost entirely out of protein, β-keratin (Murphy 1996). They have a complex structure, which differs among different types of feathers. The feathers have, during evolution, become modified to many forms, which **fulfill diverse functions**. Coverts provide a barrier against adverse environmental conditions. They can also be used for display and camouflage. Down feathers ensure insulation of the body (Ginn, Melville 1983). Probably the most obvious function of the feathers is flight, ensured by the flight feathers in wings. Their moult and properties are frequently studied (Bonser 1996; Dawson et al. 2000; DesRochers et al. 2008). The typical flight feather has in the centre a long rachis. The rachis is subject to large aerodynamics forces during flight. It should be stiff, to withstand overload during the flight, and on the other hand elastic, to avoid breaking. The vanes make the bearing surface of the wing. The vane consists of numerous barbs, attached to the rachis and connected with barbules with hooks. Barbules have to be linked tightly, to efficiently catch wind (Ginn, Melville 1983; Bonser 1996).

**Over time, during use, the feathers lose their properties.** Poor condition of the feathers alters their functions – they cannot perform them well and this handicaps the bird significantly. To not lower their chances for survival, **the birds have to replace their feathers.** The overall process of replacement of the feathers is called **moult.** Most birds, especially passerine birds, like zebra finches, replace their feathers once a year (Ginn, Melville 1983; DesRochers et al. 2008). However, **the moult is costly.** It is considered as one of the most energetically demanding activities in birds, next to migration and reproduction. These three events do not overlap significantly (Ginn 1975; Ginn, Melville 1983). Birds have to use energy to produce new feathers, regulate body temperature while feathers insulation is reduced, and overcome less flight efficiency (Bonser 1996; Murphy 1996). Basal metabolism rate (BMR) in some species is increased twice

during the moult (King 1981, after Lindström et al. 1993; DesRochers et al. 2008). Murphy and Taruscio (1995) showed that sparrows significantly increase their synthesis of proteins and tissues during moult. If the conditions are not favourable, moult can be slowed down or even stopped for a while, when feather production would compete for limited resources (Barta et al. 2006). One of such conditions may be aging.

**Aging is a "persistent decline in the age-specific fitness components** of an organism due to internal physiological deterioration" (Rose 1991). Whole organismal declines in metabolic performance are known to occur due to aging in avian species. Production of energy and synthesis of protein is lowered (Makrides 1983). We know a lot about the aging of other keratin structures, hairs. **However, there is a considerable gap in our knowledge about the feathers' moult, growth rate, and characteristics in aging birds.** Since feathers are so important for birds, recognition of possible changes in the moult and feathers' properties during senescence seems significant.

**The aim of this study is an examination of possible changes in feathers' quality and moult costs in aging birds. We will use the zebra finch (*Taeniopygia guttata*) as a model animal. Using an induced moult, we will compare the growth rate, mechanics, and structure of feathers among young and old birds. With the use of a wind tunnel and climatic chambers we will examine how particular moult costs differ in young and old birds. As a result, we will try to understand the effects of aging on the moult and the feathers' quality.**

## 2. Hypotheses

To achieve the objectives of the study, we will test the following hypotheses:

**H1.** In an induced moult, flight feathers will regrow slower in old birds than in young birds.

**H2.** Flight feathers in old birds will have poorer condition than feathers in young birds. The feather's mass (indication of amount of the protein) and length, elasticity, number of barbules per area, and resistance to degradation by bacteria will be declined in older birds.

**H3.** Costs of thermoregulation during moult are higher in old birds than in young birds.

**H4.** Costs of overcoming reduced flight efficiency due to moult are higher in old birds than in young birds.

## 3. Research methodology

**Hypotheses 1. and 2.**

We will conduct the study in the colony of captive zebra finches (*Taeniopygia guttata*) in the Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Kraków. The zebra finches are model animals, their biology is well-known. They moult feathers all year round. Also, they are available both in the Institute of Environmental Sciences and the Max Planck Institute, where the last experiment will be conducted. Study will be carried out in young and old birds. Zebra finches can live up to 5-6 years old in the wild (up to 9 years in captivity) (Zahn 1996; Moe et al. 2009), so five or six-year-old birds may be considered as old individuals. In the study, four age groups of birds will be distinguished: one-year-old, two-year-old (young birds), five-year-old, and six-year-old (old birds). There will be 20 individuals, 10 males and 10 females, in each group (80 in total).

We will pluck two selected flight feathers (one primary and one secondary) from both wings and observe their regrowth. Such manipulation is called an **induced moult** (task T1). The induced moult does not mimic the natural moult but enables to study growth rate of the feathers. During study, all birds will be kept in the same conditions in the indoor aviary. Birds will be observed for about 1.5 month, until the plucked feathers grow back. Every week in the morning, before the birds start feeding, we will check the moult of all flight feathers (moult score; Ginn, Melville 1983), measure bird's body mass and a length of regrowing feathers. The expected duration of the study and the frequency of measurements were estimated on the basis of the pilot study. When the feathers regrow, we will pluck them again for further analyses (Fig. 1.).
From the change in the feather's length over time we will examine the **speed of regrowth**.

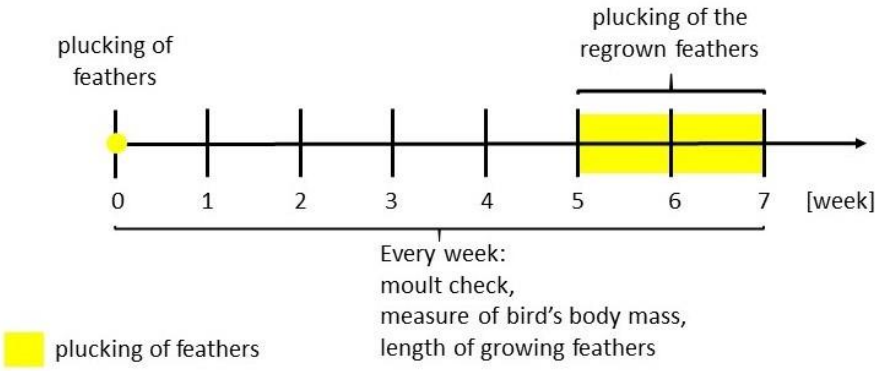


Fig. 1. Timeline of the induced moult.

The properties of the feathers from the second plucking will be studied (T2). We will measure the **mass** and the **length** of the feathers. Then, we will take pictures of the feather's vane in a fixed place with a camera mounted to a light microscope. We will measure **inter-barb distances** and count the **number of barbules** in a fixed area (see DesRochers et al. 2008). To examine **elasticity**, we will apply a fixed pressure (measured with an electronic dynamometer) to the rachis and measure the bend angle of the feather. At the end, we will use the technique described by Williams et al. (1990) for a **bacterial degradation**. We will prepare a sterilized feather medium from each feather and grow on it bacteria *Bacillus licheniformis*. Then, with the use of a spectrophotometry, we will analyse "the concentration of oligopeptide fragments released by bacterial degradation of keratin" (DesRochers et al. 2008).

**Hypothesis 3.**

The study will be conducted in the colony of captive zebra finches in the Institute of Environmental Sciences, Faculty of Biology, UJ. Again, we will distinguish the same four age groups as in the previous study, with the same number of birds, but we will use different individuals.

During the experiment, birds will be placed in open-circuit climatic chambers and exposed to four different **T*a*** (ambient temperatures) with values between 25 and 40 °C (at intervals of 5 °C) for 1 h (based on Nichelmann, Tzschentke 2002). After 1-h exposure to the respective Ta values, **heat production** (HP) will be determined (T3) by measuring of $O_2$ consumption and $CO_2$ production (see Nichelmann, Tzschentke 2002). To evaluate a contribution of changes in feathers insulation, we will measure $O_2$ and $CO_2$ levels before and after clipping of a plumage. We will trim feathers covering the pectoral muscles and abdomen at

the area of about 9 $cm^2$ with small scissors (see Schieltz, Murphy 1997; Nord, Nilsson 2018). We will measure HP in the metabolic chambers, which have a water source and are composed of transparent acrylic. They will be placed in a temperature-controlled water bath.

**Hypothesis 4.**

The study will be conducted in the colony of captive zebra finches in the Max Planck Institute for Ornithology in Starnberg, Germany. The birds had previously been trained in a wind tunnel.

Again, four age groups of birds will be created for the experiment. The age range and number of birds in each group will be the same as in the previous studies. **Flight efficiency** (T4) will be measured in the **wind tunnel** (Casagrande et al. 2020). For this purpose, the **metabolic rate** (doubly labeled water technique (Jodice et al. 2016)), the **frequency of wing flapping**, and the **response to turbulence** will be examined. We will carry out the measurements before and after reduction of length of selected primary flight feathers (like in Swaddle et al. 1996).

Obtained data will be analysed using general linear model (with age of birds in months as a covariance) with statistical software: R-Studio (version 1.3.1093 RStudio, PBC) and Statistica (version 13 TIBCO, Software Inc.).

## 4. Expected outputs

We expect that the flight feathers will regrow slower in old birds than in young birds. During aging, synthesis of protein is declined and the growth rate of the feathers in older birds will be lowered. Also, the feathers' mass and length, and the elasticity of the rachis will be declined in older birds. In older birds, the feathers will be less resistant to degradation by bacteria. Additionally, we expect that the feathers will have less barbules and they will be less dense, which can lead to a worse flight performance in older birds in a wind tunnel. The metabolic rate and wing flapping will be higher in "moulting" older birds (in comparison to young ones) to overcome reduced flight efficiency. On the other hand, the response to turbulence will be worse. Also, heat production in "moulting" old zebra finches will be higher than in young individuals. Their insulation and mechanisms of thermoregulation will be less effective which will make them produce more heat to keep constant body temperature.

## 5. Expected timescales

Our four **hypotheses** (**H1-4**) will be tested in four **research tasks** (**T1-4**). Table 1. shows how studies will be carried out over the year.

Table 1. Breakdown of research tasks (T) in time (shaded cells), with their contribution to the research hypotheses (H).

| Hypothesis | Research task | Aim | quarter of the 2021 | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| H1, H2 | T1 | Induced moult - feathers' growth rate | | | | |
| | T2 | Analysis of properties of the feathers. | | | | |
| H3 | T3 | Examination of moult costs due to the reduced insulation. | | | | |
| H4 | T4 | Examination of moult costs due to reduced flight efficiency. | | | | |
| | | Analysis of results, preparation of an open-access article. | | | | |

**Additional questions:**

**What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?**

Because of the work with birds, their feathers, and inside the indoor aviary, participants of the project will be exposed to dust and allergens from birds. People with an allergy to mites and birds' feathers, epidermis, and faeces, may develop symptoms such as asthma, allergic rhinitis and conjunctivitis (mp.pl, access on 07.12.2020). To minimise these risks, before starting the research, each participant will undergo a medical examination (a comprehensive pulmonary appointment), including spirometry and diastolic test. These tests will help to assess the respiratory fitness and check for asthma. People with decreased respiratory capacity will limit direct contact with birds. Also, all participants will wear protective clothing such as an apron, disposable gloves and possibly a mask.

**Please provide details of agreed collaborations and project partners that will facilitate the proposed project:**

Collaboration

The project will be implemented in cooperation with the Max Planck Institute for Ornithology. The institute will finance the costs of the use of a wind tunnel and enable research - in one of the three wind tunnels for birds operating in the world. In the study, we will also use the zebra finches from the colony from the Institute.

The project will be also implemented in cooperation with the Institute of Geological Sciences of the Polish Academy of Sciences. The institute will finance the costs of the use of the isotope ratio mass

spectrometric analysis (IRMS) to analyse blood samples collected using the Doubly Labeled Water (DLW) method.

Project partners

The Institute of Environmental Sciences, UJ will provide birds from the colony for the studies (T1-T3). Teams of Plant-Microbial Interactions, Physiological Ecology, and Aquatic Ecosystems from the Institute will lend us equipment and laboratories, and help us with conducting experiments and analyses. The missing equipment and reagents will be bought.

The Department of Mineralogy, Petrology, and Geochemistry, Institute of Geological Sciences, Faculty of Geography and Geology, UJ will enable us to make the spectrophotometry analysis.

**Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:**

Institute of Environmental Sciences, Faculty of Biology, UJ, in Kraków

The zebra finches colony in the institute has several hundred individuals, 1 – 6 years old, with known hatching dates. It makes it possible to study moult in birds of different ages, both in young and old individuals. Indoor aviaries allow us to keep big groups of birds in the same climatic conditions. Special open-circuit climatic chambers make it possible to keep birds in precise temperatures with values between 25 and 40 °C. Also, the Magnos 4 oxygen analyser will enable us to measure oxygen consumption in single birds.

The laboratories of the Plant-Microbial Interactions Team, Institute of Environmental Sciences, UJ, will provide us conditions for growing bacteria on a feather medium. We will measure the concentration of oligopeptide fragments released by bacterial degradation with a spectrophotometer in the Department of Mineralogy, Petrology, and Geochemistry, Institute of Geological Sciences, Faculty of Geography and Geology, UJ. Finally, the Aquatic Ecosystems Team, Institute of Environmental Sciences, UJ, will enable us to use a microscope with a mounted camera to take pictures of the feather's vane.

Max Planck Institute for Ornithology in Starnberg, Germany

The Max Planck Institute for Ornithology has two departments and several independent research groups that investigate different ornithological topics by using an interdisciplinary approach. A special feature of the institute is the wind tunnel which was specially made to study physical and physiological problems related to birds as it allows researchers to actively observe them in the flying section. Natural flight conditions can be simulated in the tunnel by varying wind speeds, air pressure, and temperatures, and via projections of landscape and sky.

Institute of Geological Sciences of the Polish Academy of Sciences

The Stable Isotope Laboratory has the equipment to analyse the stable isotopic composition of N, C, O, and H (including deuterium) in all compounds. The analysis is provided by the GC IsoLink + Trace GC ultra with IsoLink GC Thermo Scientific connected to IRMS MAT 253, Thermo Scientific.

**Please provide details of necessary permits/licences obtained, if applicable:**

To conduct research on laboratory animals, including plucking of feathers and injecting birds with doubly labeled water, we will need consent of the ethics committee (Krajowa Komisja Etyczna ds Doświadczeń na Zwierzętach). The need to obtain a permission results from the resolution "USTAWA z dnia 15 stycznia 2015 r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych". Only people who completed the course and have an assignment for performing experiments on animals will be directly involved in conducting procedures on animals.

We will pluck or trim only two flight feathers from each wing, which is 10.5% of all flight feathers (19 in total). It should not badly affect birds, as they live in captivity and will not need to fight for food, feed young, or escape from predators. Also, trimming of contour feathers should be safe for the birds. The same procedure was done on the wild birds during breeding season (Nord, Nilsson 2018), so it should have even more benign effects on the captive birds.

**Please indicate how you will assess the scientific impact of the project and the benefits for non-academic audiences:**

There is a growing interest in aging in science. In the last 10 years, the number of publications about aging doubled (Data Citation Index [Web of Science]; Google Scholar). We know a lot about the aging of hairs in mammals, especially humans. However, there is a considerable gap in knowledge of how aging affects feather's quality and moult costs. There are no studies about the effects of aging on the feathers and the moult. Many research about moult and feather's quality were carried out in the field, on the wild birds, which made it impossible to study the effect of aging. In most wild birds scientists cannot set the precise age of birds. This study will be the first to present the effects of aging on the moult costs and the feathers' quality. It will combine many aspects of the effect of aging, as we will study different characteristics of the feathers and two different costs of moult. Our results will help to fill the knowledge gap. If the results are promising, they can also indicate the direction of further research about the feathers, moult, and aging. We will study only one species of bird, in captivity - next studies could focus on wild birds from breeding colonies, like on Gotland in Sweden, or provide information on other species, to create a more general picture.

As basic research, this project may not have direct benefits for non-academic audiences. However, it can be applied in some veterinary studies on the birds kept in the home as pets, or as poultry. Birds are popular pets and their owners should care about their welfare. Knowledge about how aging affects birds during moult and the quality of their feathers may help maintain the proper quality of birds' life. If we know the particular negative impacts of aging on birds, we will be able to study them more and minimise them.

**How do you intend to make your research data publically available?**

We will publish our findings in a scientific journal focusing on ecology or zoology. It will be the first study of the impact of aging on moult and feather's quality. Topic is innovative, combines many aspects, and will bring new knowledge to bird ecology and aging. The research data will be published in journals of impact factor between 1.6 (*Journal of Zoology*) and 8.7 (*Ecology Letters*), depending on the quality of

obtained results. Research similar to ours were published in such journals. We will publish one open access article (Wiley. Open access. Article Publication Charges (APCs)).

**Please provide details of any published papers relevant to this project:**


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## Budget

Equipment

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| Electronic dynamometer with an internal sensor | 1 | dynamometer from SAUTER FK 50 | 1 030 PLN |
| Temperature and humidity recorder | 2 | temperature and humidity recorder with an internal sensor (NTC) and a capacitive humidity sensor | 1000 PLN |
| Hamilton RN syringe 250-1000 µL | 2 | 700 Series Microliter Syringes (with replaceable needle)<br>Hamilton | 990 PLN |

| | |
|---|---|
| Overall Equipment Cost: | 3 020 PLN |

Consumables

| Item | Quantity | Description | Total Cost |
|---|---|---|---|
| *Bacillus licheniformis* ATCC® 12759™ | 1 | Strain of bacteria *Bacillus licheniformis* (Pol-Aura) | 1 200 PLN |
| Nonselective medium for *Bacillus* | 1 | CASO Agar for microbiology, NutriSelect™ Plus 500g | 500 PLN |
| Petri dishes | 1 | 1 set of sterile Petri dishes | 430 PLN |
| Inoculation loops | 1 | 1 set of inoculation loops | 200 PLN |
| Paper towels | 1 | 1 box of 100 paper towels sets | 100 PLN |
| One time masks | 10 | 10 boxes of 50 one time masks | 300 PLN |
| One time gloves | 30 | 30 boxes of 100 one time gloves | 2000 PLN |
| Capillaries for blood collection | 1 | 1 set of 100 capillaries for blood collection | 50 PLN |
| Needles for Hamilton RN syringe 250-1000 µL, 22ga | 12 | 12 boxes of 6 needles | 7 350 PLN |
| Deuterium Oxide | 1 | 100ml | 1 350 PLN |
| Blood collection needles Neonatal 23 G | 2 | 2 boxes of 50 needles | 610 PLN |
| Overall Consumables Cost: | | | 14 090 PLN |

Personal and Field Travel/Accommodation/Subsistence

| Description | Total Cost |
|---|---|
| Diesel (for 1870 km with fuel consumption of 5.7 l per 100km) | 600 PLN |
| Private car | - |
| Highway fee | 200 PLN |

| | |
|---|---|
| Accommodation (for one month, for 2 people) | 4 920 PLN |
| Catering (for one month, for 2 people) | 7 900 PLN |
| Overall Travel Cost: | 13 620 PLN |

Employment (note only casual, short term assistance will be considered)

| Position | Description of role | Rate & Duration of employment | Total Cost |
|---|---|---|---|
| Principal Investigator 1 | Master student with an assignment for performing experiments on animals | 3 000 PLN per month, 12 months | 36 000 PLN |
| Principal Investigator 2 | Master student with an assignment for performing experiments on animals | 3 000 PLN per month, 12 months | 36 000 PLN |
| Principal Investigator 3 | Master student without an assignment | 3 000 PLN per month, 9 months | 27 000 PLN |
| Principal Investigator 4 | Master student without an assignment | 3 000 PLN per month, 9 months | 27 000 PLN |
| Overall Employment Cost: | | | 126 000 PLN |

Other costs (including overheads)

| Description | Total Cost |
|---|---|
| Comprehensive pulmonary appointment for 4 participants (Unimed) | 600 PLN |
| Overheads (use of zebra finches colony, laboratories, and equipment) | 75 000 PLN |
| Open access article | 15 000 PLN |
| Overall Cost: | 90 600 PLN |

| | |
|---|---|
| Total project cost | 247 300 PLN |