GRANT WRITING IN ECOLOGY AND ENVIRONMENTAL CONSERVATION

13-18 September 2012 | www.eko.uj.edu.pl/phd OCHOTNICA GÓRNA FIELD STATION 2012

nejczer

Course co-financed by the European Union under the European Social Fund

HUMAN CAPITAL
NATIONAL COHESION STRATEGY

EUROPEAN UNION
EUROPEAN SOCIAL FUND

CONTENTS:

1. *WORKSHOP PARTICIPANTS*

1 **Ewa Chmielowska**; *Department of Anthropology, Institute of Zoology, Jagiellonian University;*
Ewa.chmielowska@uj.edu.pl
2 **Justyna Gutowska**; *Nature Conservation and Environmental Education Research Team, Institute of Enviromental Sciences, Jagiellonian University;*
justyna.gutowska@uj.edu.pl
3 **Damian Kolbe**; *Department of Entomology, Institute of Zoology, Jagiellonian Universtiy;*
damian.kolbe@uj.edu.pl
4 **Michał Kuciel**; Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University*;*
m.kuciel@uj.edu.pl
5 **Diana Maciąga**; *Botanic Garden, Institute of Botany, Jagiellonian University ;*
diana.maciaga@uj.edu.pl
6 **Joanna Sudyka**; *Population Ecology Group, Institute of Environmental Sciences, Jagiellonian Universtiy;*
joanna.sudyka@uj.edu.pl

2. *ORGANIZATORS AND REVIEWERS*

- Organizator:

Prof. dr hab. Mariusz Cichoń, *Population Ecology Group, Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; mariusz.cichon@uj.edu.pl*

- Reviewers:

Prof. dr hab. Adam Łomnicki, *Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; adam.lomnicki@uj.edu.pl*

Dr Beata Klimek, *Environmental Monitoring Group, Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland;* beata.klimek@uj.edu.pl ;

Dr hab. Paulina Kramarz, *Life History Evolution, Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; paulina.kramarz@uj.edu.pl*

Editor of the Report: Ewa Chmielowska

3. RESEARCH TOPICS SUGGESTED BY PARTICIPANTS

1) Biodiversity and frame of mind
2) Caloric restriction and the lifespan in insects
3) Does the wolf-dog hybridization increase the risk of the livestock predation
4) The influence of oxygen concentration on growth rate in two fish species differing in tolerance of oxygen availability
5) The influence of water pollution on insect biodiversity
6) The influence of pearl powder on skin inflammation
7) The influence of local food on health status
8) Sex ratio in early and late broods in birds
9) Seedling density in relation to spruce forest management
10) The effect of different oxygen concentration on the development of gills in amia calva and cyprinus carpio
11) Insect species feeding on invasive plants
12) Accumulation of chloroxylenol in fish liver
13) Environmental education at childhood and adult life environmental attitudes
14) Function of fur color in urban cats
15) Crop losses caused by wild boar in relation to the intensity of hunting
16) Efficiency of effluent treatment in terms of drug metabolites
17) Toxin resistance in cockroaches
18) Olfactory attractiveness of male rat

4. PROJECTS AND REWIEVS

4.1. The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake

***A*uthors:** Michał Kuciel, Joanna Sudyka

4.1.1. First version of the project

Project summary

Bowfin (*Amia calva*) is a lungfish able to breathe air employing its swim bladder as a lung when there are lacks of oxygen dissolved in water while carp (*Cyprinus carpio*) can only use gills for oxygen intake. Hyperoxia and hypoxia are considered to be stress factors in the aquatic environment and each of the species should response differently to such aggravated conditions. We plan to examine influence of various oxygen concentrations on important life-history trait which is growth rate and dynamics. We will place 100 fish of single species, distributed equally in four tanks, in three oxygen levels (hyp-, norm- and hyperoxia). After six months we will compare influence of treatments on growth rate and growth dynamics between species. We expect that bowfin's weight should be less affected by hypoxic conditions. If not, then it would denote that the role of lung breathing is not enough for compensation. Albeit it is not clear how freshwater species react to prolonged hyperoxia from the earliest stages of development. Our study would be first in this field.

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

1. *What problem is being proposed and why?*

Fish are considered to be most suitable models to study oxygen dependent processes due to their acclamatory or adaptive responses with respect to their requirements (Lushchak & Bagnyukova 2006, Nikinmaa 2002, Soitamo et al. 2001). The aim of the experiment is to test whether differences in relative growth rate and growth dynamics between the species differing in oxygen intake mechanism in various oxygen conditions (hypoxia, normoxia, hyperoxia) exist. Hypoxia and hyperoxia can occur naturally (van Raaij et al. 1994; Nikinmaa 2002) so such experimental design corresponds with natural problems. Since bowfin (*Amia calva,* Linnaeus 1766) is a lungfish and has an ability to extract atmospheric oxygen we presume that it will be less affected by reduced water oxygen concentration. Carp (*Cyprinus carpio*, Linnaeus 1758) however

should suffer from limited oxygen availability as it cannot compensate for this lack (Mustafa et al. 2011). Until now there have been no studies concerning prolonged influence of hyperoxic conditions on fish (Wu 2002). Furthermore there is no data concerning impact on fish from the earliest stages of life neither for hypoxia nor for hyperoxia.

The key point of this study is to breed a juvenile specimens having unlimited food access in different oxygen conditions and measure their body mass. We predict that bowfin's growth rate under hypoxic conditions will not be much lower than its growth in normoxic conditions, what will show that breathing by the lungs is compensating oxygen deficiency. If the growth rate of bowfin is lower due to hypoxia, then it would mean that breathing by lungs is not efficient enough or energetically costly. We already know that hypoxia affects growth for carp, but it had never been checked how it works at early stage of life for such a long period. If there will be differences in growth for hyperoxic conditions it may denote that excess of oxygen is harmful enough to affect fish growth.

2. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

Fish are most numerous and one of the most diversified vertebrates. Common limiting factor in water habitats is oxygen. Many fish species have different adaptations to manage the problem of hypoxic conditions (Sayer 2005). Carp is characterized by high tolerance for low oxygen concentration in water. It can administer oxygen efficiently in the period of hypoxia (or even anoxia) for example by accumulation of high amounts of glycogen in brain (Vornamen & Paajen 2006). Bowfin is a species which also can survive in a water with low oxygen level, but the mechanism is significantly different. It has a swim bladder transformed to lung what makes it possible to breath an air, when using gills is not sufficient (Daxboeck et al. 1981).We expect that bowfin should cope better with poor oxygen conditions because of that diversified possibilities.

It is known that hypoxia and hyperoxia affect carp's blood plasma activity of glutathione peroxidase, an enzyme protecting organism from oxidative damage, there was 37% increase of activity in hyperoxia and 38% decrease in hypoxia. The oxidative DNA damage was higher in both hypoxic and hyperoxic conditions. Also hematological parameters showed enhanced values under hypoxic conditions. The damage to liver and gills occurred due to both treatments. The specific growth rate was also affected in hypoxic conditions by 30% and was correlated with DNA damage. Prolonged hypoxic and hyperoxic conditions are responsible for oxidative stress

responses at DNA and tissue levels (D'Aoust 1969, Mustafa et al. 2011). However until now experiments used only short term exposures (up to 30 days) and were performed on developed fish. There was no study attempting to check influence shortly after hatching for initial growth process which is crucial for organism. Moreover there has been no research of this kind for bowfin at all. While carp's growth rate decreases during low oxygen concentration (Mustafa et al. 2011) we want to compare it with the bowfin's growth rate to estimate the effectiveness of this two species growth in different oxygen conditions. The differences between species with distinctive oxygen intake could enhance our reasoning concerning oxygen scarcity as limiting factor for growth.

3. *What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?*

In our experiment 300 carps and 300 bowfins will be distributed randomly into twenty four 100 l water tanks (25 fish of single species in each). This way each species and each treatment will be present in four tanks (Tab. 1). The methodology of achieving experimental oxygen conditions were followed after Mustafa et al. 2011. Normoxia (control group) at 7.1 ± 1.04 mg O_2 l^{-1}, hypoxia at 1.8 ± 1.1 mg O_2 l^{-1}will be achieved by pumping 99,99% nitrogen gas and hyperoxia at 12.3 ± 0.5 mg O_2 l^{-1}will be created by pumping oxygen of 99,95% purity. The oxygen concentration shall be measured daily with oxygen electrode (Oxy Guard, Handy Polaris, DK). Water temperature will be maintained at 20°C and pH at 6,5-8,0 while photoperiod 12h light/12h dark. Fish with visible defects will be removed from the tanks. Experiment will last for six months and fish will be automatically fed with automatic feeders (*Naididae*, *Lumbricidae* worms and *Chironomidae* flies plus vitamin supplementation) every four hours to ensure unlimited food access for every individual. To eliminate all types of contamination (faeces, bacteria, food remains) active filtration (mechanical, biological and UV treatment) will be applied. Fish will be weighted every three days (average fish weight from each tank).

Fertilized bowfins' eggs will be purchased (there is no need for special permissions) from breeding lab at School of Environment and Natural Resources, Ohio State University (transport under permanent aeration) and the carps' fertilized eggs from local suppliers. During the first 21 days of life (until bowfin is fully capable of atmospheric oxygen usage (Ballard 1986, Dąbrowski, pers. comm.)) fish will be kept in aquariums with normoxic conditions and fed with *Artemia*. After experiment termination fish could be turned over to zoological gardens as animal fodder.

Tab. 1. A schematic illustration of breeding set of tanks. In each tank there will be 25 specimens

	Hypoxia		Normoxia		Hyperoxia	
Cyprinus carpio	HYP 1C	HYP 2C	NORM 1C	NORM 2C	HYPER 1C	HYPER 2C
	HYP 3C	HYP 4C	NORM 3C	NORM 4C	HYPER 3C	HYPER 4C
Amia calva	HYP 1A	HYP 2A	NORM 1A	NORM 2A	HYPER 1A	HYPER 2A
	HYP 3A	HYP 4A	NORM 3A	NORM 4A	HYPER 3A	HYPER 4A

Whether influence of treatment on growth rate (initial weight vs. final weight) is different between species (Fig. 1) shall be assessed by interaction. We will employ ANOVA with both factors fixed.
$GR=\mu+S+O+(SO)+e$
(GR-growth rate; S-species; O-oxygen conditions)

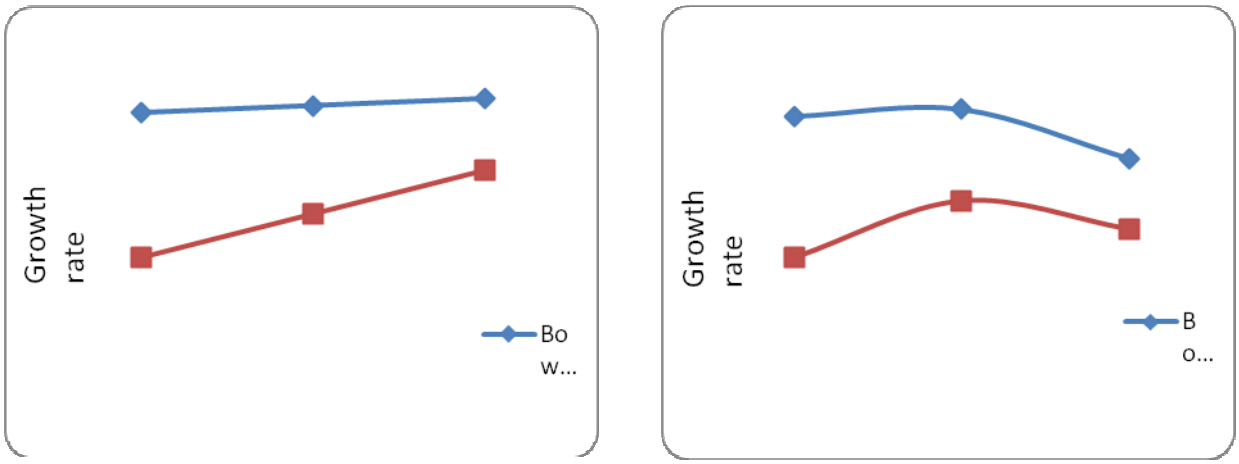

Fig. 1. Expected outcome of treatment on growth rate

To assess dynamic of weight gain growth curves will be created (on the basis of all measurement) and their parameters will be compared. After transforming the data repeated measure ANOVA will test for existing differences among curves.

4. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

Our results may be beneficial for understanding role of limiting factor - oxygen concentration on crucial life-history trait which is growth dynamics. It may help avoiding errors in commercial fish farming and it may appear if bowfin's breeding would be more economical in low oxygenated ponds. We plan to share our findings on international forums by means of articles in biological journals and participation in at least two conferences such as World Fisheries Conference, The International Congress on the Biology of Fish or Fisheries and Marine Sciences Conference.

References

Ballard W. W. 1986. Stages and rates of normal development in the holostean fish, *Amia calva*. J. Exp.Zool. 238 (3): 337–354.

D'Aoust B.G. 1969. Hyperbaric oxygen: toxicity to fish at pressures present in their swimbladders. Science 163 (3867): 576-578.

Daxboeck C., Barnard D.K., Randall D.J. 1981. Functional morphology of the gills of the bowfin, Amia calva L., with special reference to their significance during air exposure. Respir. Physiol. 43(3): 349-364.

Lushchak V.I., Bagnyukova T.V. 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol 144: 283–289.

Mustafa S.A., Al-Subiai S.N., Davies S.J., Jha A.N. 2011. Hypoxia-induced oxidative DNA damage links with higher level biological effects including specific growth rate in common carp, *Cyprinus carpio* L. Ecotoxicology 20 (6): 1455-1466.

Nikinmaa M. 2002. Oxygen-dependent cellular functions—why fishes and their aquatic environment are a prime choice of study. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133:1–16.

Sayer M.D.J. 2005. Adaptations of amphibious fish for surviving life out of water. Fish and Fishereies 6: 186–211.

Soitamo A.J., Rabergh C.M.I., Gassmann M., Sistonen L., Nikinmaa M. 2001. Characterization of a hypoxia-inducible factor (HIF-1a) from rainbow trout. Accumulation of protein occurs at normal venous oxygen tension. J. Biol. Chem. 276: 19699–19705.

van Raaij M.T.M., Breukel B.J., van den Thillart J.M., Addink A.D.F. 1994. Lipid metabolism of goldfish, (*Carassius auratus* L.).Hypoxia-induced oxidative DNA damage during normoxia and anoxia. Indications for fatty acid chain elongation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 107: 75–84.

Vornanen M., Paajanen V. 2006. Seasonal changes in glycogen content and Na+-K+-ATPase activity in the brain of crucian carp. Am. J. Physiol. Regul. Integr. Comp. Physiol. 291(5): 1482-1489.

Wu R.S.S. (2002) Hypoxia: from molecular responses to ecosystem responses. Mar. Pollut. Bull. 45: 35–45.

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yy)	Expected cost (zł)
1	The purchase of equipment	04.2013	26710
2	The purchase of fish	05.2013	6900
3	Fish breeding	12.2013	19963

4	Data processing	03.2014	8200
5	Preparation of papers and conference participation	09.2014	10000
		Total	71773

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)		
		2013	2014	Total
1	Direct costs, including:	41210	14000	55210
	1/ Salaries and benefits	9000	4000	13000
	2/ Equipment	21710	0	21710
	3/ Other direct costs	10500	10000	20500
2	Indirect costs	12363	4200	16563
3	Total costs (1+2)	53573	18200	71773

Details of direct cost items

1/Salaries and benefits
Project managers salary : 10 000 zł
Technical staff salary: 3000 zł
2/Equipment
350 bowfins: 1750 zł
300 carps: 150 zł
24 100 l fishtanks: 3500 zł
24 thermostat heaters 100W: 1450 zł
24 automatic feeders, 3600 zł
24 filters with UV lamp, 6000 zł
24 termometers: 150 zł
weight: 200 zł
10 plastic containers for fish weighting: 50 zł
net: 10 zł
hygienic materials (gloves, paper towels, protective cloathing): 250zł
1 oxygen electrode Oxy Guard: 4500 zł
1 pH meter 100 zł
3/ Other direct costs
fish food and vitamin supplements: 5000 zł
bowfin's transportation: 5000 zł
books and journals: 500 zł
conferences (for 2 people): 10000 zł

4.1.2. Rewievs

- Adam Łomnicki

Review of the grant proposal: "The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake"

All the required elements of the grant proposal are given in it, however there are many not very clear points and some confused statements. I have marked them by the comments in the electronic version of the proposal and this commented version is send with the Review.

It is not clear for me what for this problems has to be studied. The statement that nobody up to now has studied the effects of the shortage or the excess of oxygen in these two species is not sufficient to do such studies. There are several thousands of factors affecting organisms and several millions of organism in which the effects of these factors can be studied. The are millions of relations which were never studies. What is required in an ambitious project, which is of importance for the development of biological theory or for some economic applications. The argument that these project is of importance for fish farming is not convincing. I am not sure whether there is any commercial farming of bowfin and I can hardly believe that the comparison of these two species will improve commercial farming of carp, I know that the authors of this proposal were limited by the shortage of time and they have no access to full scientific literature but it should not prevent them from making an ambitious project. This is the main problem of Polish researchers: they are not ambitious enough and they are happy to carry on studies of little importance.

I was unable to find a place in the proposal in which the hypothesis or hypotheses are clearly and univocally stated with the alternative hypotheses. I also expect in the grant proposal the presentation of theoretical or practical consequences of the hypotheses presented as well as their alternatives. I should be clearly stated what is the outcome of the experiments which would make us to accept one hypothesis and what is the outcome which would make us to accept its alternative and then the further important consequences of these different results. If the consequences are not important it does not make sense to spend young age on making unimportant investigations.

Some questions given in the instruction for reviewers were answered above. The answer to other does not seem important taking into account that hypotheses, their relations to the outcome of experiments and its important consequences are not presented. I have never worked in with laboratory fish population therefore I do not know whether the details of the arrangement of the

laboratory procedures are the proper one, but even if they are not, the authors are justified to make the errors. It is because they have no access in many laboratories in the city of Krakow in which they can obtain some advices how to carry on such experiment. The most important in the grant proposal is to express clearly what we want to do, what are the consequences of our work and why it is so important that it is worth to spend two years of young age to do it.

- Paulina Kramarz

The project addresses crucial and still not well explored problem of availability of oxygen for living organisms and its influence on some life history traits. At the same time, despite of properly defined scientific justification of proposed study, clarity of the proposal and its proper structure (only keywords are missing) I have some remarks that may make it more successful.

1. Study species

Correctly chosen vertebrate taxon representing the most diverse mechanisms of oxygen uptake (among vertebrates, not all Animalia Kingdom, what should be stressed). Furthermore, the Authors focus only on freshwater species what allowed them to escape from influence of osmotic properties of environment on the studied traits.

The weak point of taking into account only two species is that differences in obtained data can come not from species response to studied factor but simply because they are different species. Only when the same pattern would be found in the range of species revealing described in the proposal mechanism of oxygen uptake conclusion on either advantage or disadvantage for fish of having lungs could be made. Consequently, the number of each species replications should be replaced by less replications but more species – at least 10 (see papers by Garland T., Jr). I advise to study the species of Teleostei which do not use swim bladder to respiration and compare them with some representatives of Lungfish. The bowfin is represented only by one species - it could be included in the study but keeping in mind its exceptional way of oxygen uptake.

2. Experimental design

There are two weak points. Firstly, instead of the response of many, one individual of each species should be measured. Otherwise you cannot control density effects – like some chemical clues coming for example from feces. One individual design allow you to reduce the size of experimental containers and to save money for purchasing/obtaining more species (see above). The only way to measure the response of a group of individuals (if there is any justification of doing so) is to estimate before an optimal density – to skip the density problem.

The second weak point is the chosen temperature -is it optimal for both species? Oxygen availability for ectotherms is strongly related to the external thermal conditions. The best solution would be to measure the response in an optimal temperature for given species and in one common within the range of thermal optimum for all species. But it would complicate the experimental

design based on more than one species – in this case I would advise to employ only one temperature, equals the comfortable one for all species.

3. Data analysis

I am not sure if I understand well differences between relative growth rate and growth dynamics – it should be more precisely explained, also the way of the calculating (Bertalanffy equation, for example?) and analyzing the outcomes. Consequently, depending on the Authors explanations, the repeated measures ANOVA will be or will not be justified. Proposed statistical analysis together with the way and the reason of showing figure 1 (as it is not well described) are rather blurry.

Please remember that if you would follow my suggestion in regards of extending of species number, then in statistical analysis phylogenetics correction should be taking into account (again, see papers by Garland, T. Jr).

4. Equipment

The experiments should be made in constant temperature conditions – despite of chosen thermal regime. And because ectotherms strongly react to any change in temperature, even so low as 0.001 ®C, the only way to do it is to use water baths (replacing thermostat heaters proposed by the Authors) – thus they should be included in the budget. Furthermore, both, temperature and oxygen concentrations should be controlled constantly, that means that for each experimental box separate sensor should be applied, together with sensors controller connected to the gases suppliers, not only one oxygen electrode, as it is stated in the proposal.

5. Budget

Despite of the above remarks is properly calculated.

6. Sum up

Language – clear, only some mix of tenses is made in the second paragraph of part 2.

Topic – exciting, especially because of taking into account juvenile animals. It would be even more exciting to follow the rest of life history traits of animals exposed to the proposed by the Authors conditions - how oxygen conditions during development influences the rest of the life history , depending of physiological prerequisites.

- Ewa Chmielowska

Dear Researchers,

The presented project is carefully written and fulfills presented requirements. It consists of all necessary parts, presents clearly the research problem, and presents a coherent logical model for hypothesis testing. However, there are some questions I would like to raise.

I like the clear and concise abstract, but I would change the order of two first sentences:

(2-more specific)Bowfin (Amia calva) is a lungfish able to breathe air employing its swim bladder as a lung when there are lacks of oxygen dissolved in water while carp (Cyprinus carpio) can only use gills for oxygen intake. (1- more general) Hyperoxia and hypoxia are considered to be stress factors in the aquatic environment.

What does it mean to distribute fish equally?

In the first sentence of problem description there should be acclimatory instead of acclamatory, I guess.

Concerning the breeding regimen- what are the breeding recommendations for these two different species? As far as I know (Wikipedia) bowfin is an active piscivore predators, while omnivorous carp's diet includes plants scavenged from the benthos. What if carp's diet must include plants for normal development?

When you treat both species with identical normoxia, while these two species have different optimum for oxygen level, you risk, that this will trigger an adaptative mechanisms in one or both control groups. It is manageable from statistical point of view, but is not desired for valuable biological results.

Why fish with visible defects will be removed from tanks? Those defects may be also the stress response results, and may influence further growth indirectly. I would suggest to remove only dead subjects, and consider death rate as important information.

These two species may display different adaptations to hypoxic conditions. For example, one species may have larger reduction in whole body mass/length, but relatively smaller damage in DNA and other structures. Another one may have no such regulatory mechanism, and visible growth reduction may be a result of such damages. Thus, the measure of body weight/length may be insufficient to measure stress level. How would you test such possibility (If you think it is possible at all?)

If you only need the average weight of fish in one tank, then why don't you weight whole tank then?

There is a lot of different commercial subspecies and breeds of carp, I assume the local suppliers will have a variety of eggs, how will you choose your model animal?

I am worrying that such long trip will deteriorate the quality of bowfin eggs. Maybe carp eggs should be exposed to similar conditions to equalize chances?

Figure 1 presents 2 possible outcomes of this experiment, you should modify the below description, because it is unclear

In summary- this project is formally well written, probably the best from all 3 of us, but there is a serious question about model animal choice, and treating two distinct species with the same conditions.

- Justyna Gutowska

Review of the grant proposal "The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake"

The project concerns important scientific problem. Results of the proposed study can bring some knowledge that might be useful for fish production sector.

The problem, hypothesis and predictions are clearly stated, however it is not clear why the authors predict harmful effect of hyperoxic conditions in the text ("If there will be differences in growth for hyperoxic conditions it may denote that excess of oxygen is harmful enough to affect fish growth"). At the same time, two different predictions are presented on the graphs – positive as well as negative effect of the excess of oxygen.

The information that "Until now there have been no studies concerning prolonged influence of hyperoxic conditions on fish" is supported by citation of the article from published in 2002. It's already 2012, so it definitely should be carefully checked, whether the state of knowledge have improved. Stating that "The damage to liver and gills occurred due to both treatments" the authors should refer to some studies held on this topic.

It is not clear what it means "visible defects" in the sentence "Fish with visible defects will be removed from the tanks". It is not considered, in the project description, what is the probability that the fish will suffer from some diseases or die during the experiment (e.g. because of the experimental treatment). This might be also important for planning to "turn over" the fish to zoological gardens as animal fodder after the experiment.

The research proposal is complete and written in proper, communicative language, even enriched with the table and graphs presenting experimental design and potential results of the study. The only detail missing is that the authors do not specify in which biological journals they would see their articles presenting results of the study.

There are some mistakes in the proposed budget. Materials were classified as equipment (e.g. fish, hygienic materials). At the same time, indirect costs were estimated also from the equipment value.

The project has the potential to address the scientific problem, which is important and was not studied before. However, it can be improved by eliminating few inaccuracies – the most important thing is revision of the budget.

- Damian Kolbe

Review of project: "The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake"

I don't know too much about fishes so I'm little scarred to reviewing this grant proposal but I will try. For the fist page of text in verse introducing the leaders of the project there is two things missing: coma (between application names) and institutions that they representing. By the way topic of the proposal is clearly and show us experimental contex. Summary is clearly, not too long and contain information about all the project most important points. Reading this text there is an impression that the applicants have widely knowledge about presenting problem. It's good to know that their study "would be first in this field", it's sounds very innovating.

The hyphothesis and metods are clearly presented. The project contains very usefull solutions, for example: purchase fertilized bowfins eggs to reduce sending costs or give the fish to the zoo as animal fooder after the experiment. On other way I think that results it's seems preductible too much and I have doubt if hiperoxia may exist in natural environment (if not why the fishes are tested in abnormal conditions??) I don't understand this necessity. Statistical methods, references (excluding some species names without italicized) and expected results looks fine. In last point of the grant proposal - budget there is a ambiguity: there's no need to fill the third line in proposed budget (table), it's generate error in total costs!!!

Summary I think the project is constructed very well and have chance to get succeed. Good luck wishes in the scientific career.

- Diana Maciąga

Project Assessment
The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake.

Title:

The title corresponds well with project aims. However, I would recommend using the word method instead of way.

Summary:

The summary is well planned and lucid. The assumptions of the hypothesis are clearly expounded, as well as the expected results of the experiment which are complemented by the possible explanation of their alternative outcome. The basics of the experiment is intelligibly described. The fact that the study will be the first in the field enhances its importance, however I would recommend simply calling it pioneering.

Discussed problem and present state of knowledge:

As in the case of the summary, this parts are well organized, clear and logical. The authors provide the reader with information sufficient to understand the project aims and to support the rationale for conducting the experiment.

The hypothesis is clearly presented. Various possible results are taken into consideration and are a consequence of predictions.

The lack of certain data relevant to the problem as well as the fact that the experimental conditions correspond with the naturally occurring ones acts as a supporting factor for conducting the experiment.

The experiment:

That the methodology has been successfully used in other study excludes doubts about its usefulness. The presentation of the experiment protocol in the form of a table greatly enhances its understanding.

The only question that comes to mind is how will removing fish with defects (decrease in number of the sample) affect the analysis.

The presentation of the model eliminates the possibility of preliminary errors in data analysing process.

Covering most possible aspects of the experiment, such as permissions and the lot of fish used after the end of research adds up to the project reliability and confirms an in depth planning process. Raising the question of ethics would enhance the overall impression even more.

Expected results:

The results of the project are convincingly shown as beneficial in terms of supplementing biological knowledge as well as their practical use.

The concrete plan of how the research results will be disseminated confirms authors' serious attitude towards the significance of the project.

References:

The bibliographical notes are neatly organised, consistent style is maintained, all the given papers are cited in the text.

Costs:

Fish and disposable materials should not be defined as equipment.

Language used:

The authors are lucid writers, carefully planing the project proposal in terms of a chain of causation. The language is rich yet succinct. There are no errors that would hinder understanding of the text. Only few minor mistakes were found.

Conclusion:

The project seems carefully thought over and free of errors in its logic, the proposal is very well written. For all the reasons mentioned in the assessment I find the project worth being given the needed founds.

4.1.3. Final Version Of The Project

Project Title:

The influence of oxygen concentration on growth rate in two fish species differing in the way of oxygen intake

Applicants:

Michał Kuciel: Department of Comparative Anatomy, Institute of Zoology;

Joanna Sudyka: Population Ecology Group, Institute of Environmental Sciences

Project summary

Bowfin (*Amia calva*) is a lungfish able to breathe air employing its swim bladder as a lung when there are lacks of oxygen dissolved in water while carp (*Cyprinus carpio*) can only use gills for oxygen intake. Hyperoxia and hypoxia are considered to be stress factors in the aquatic environment and each of the species should response differently to such aggravated conditions. We plan to examine influence of various oxygen concentrations on important life-history trait which is growth rate and growth dynamics. We will place 100 fish of single species, distributed equally in four tanks, in three oxygen levels (hyp-, norm- and hyperoxia). After six months we will compare influence of treatments on growth rate and growth dynamics between species. We expect that bowfin's weight should be less affected than carp's weight by hypoxic conditions. If not, then it would denote that the role of lung breathing is not enough for compensation. Albeit it is not clear how freshwater species react to prolonged hyperoxia from the earliest stages of development. Our study would be first in this field.

Keywords: bowfin, carp, hyperoxia, hypoxia, growth rate

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

1. What problem is being proposed and why?

Fish are considered to be most suitable models to study oxygen dependent processes due to their adaptive responses with respect to their requirements (Lushchak & Bagnyukova 2006, Nikinmaa 2002, Soitamo et al. 2001). The aim of the experiment is to test whether differences in relative growth rate and growth dynamics between the species differing in oxygen intake mechanism in various oxygen conditions (hypoxia, normoxia, hyperoxia) exist. Hypoxia and hyperoxia can occur naturally (van Raaij et al. 1994; Nikinmaa 2002) so such experimental design corresponds with natural problems. Since bowfin (*Amia calva*, Linnaeus 1766) is a lungfish and has an ability to extract atmospheric oxygen we presume that it will be less affected by reduced water oxygen concentration. Carp (*Cyprinus carpio*, Linnaeus 1758) however should suffer from limited oxygen availability as it cannot compensate for this lack (Mustafa et al. 2011). Until now there have been no studies concerning prolonged influence of hyperoxic conditions on fish (Wu 2002). Furthermore there is no data concerning impact on fish from the earliest stages of life neither for hypoxia nor for hyperoxia. Our results can give answers to theoretical biological problem and constitute basis for further physiological studies.

The key point of this study is to breed a juvenile specimens having unlimited food access in different oxygen conditions and measure their body mass. We predict that bowfin's growth rate under hypoxic conditions will not be much lower than its growth in normoxic conditions, what will show that breathing by the lungs is compensating oxygen deficiency. If the growth rate of bowfin is lower due to hypoxia, then it would mean that breathing by lungs is not efficient enough or energetically costly. We already know that hypoxia affects growth for carp, but it had never been checked how it works at early stage of life for such a long period. If there will be differences in growth for hyperoxic conditions it may denote that excess of oxygen is harmful enough to affect fish growth. The dynamics of growth will be shown on growth curves to see exactly how growth was affected by given conditions at every stage of studied period. It may occur that the youngest fish suffer most damage and after gaining some weight they are mature enough to cope better with various oxygen levels. It may indicate which period is the most sensitive for fish growth.

2. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

Fish are most numerous and one of the most diversified vertebrates. Common limiting factor in water habitats is oxygen. Many fish species have different adaptations to manage the problem of hypoxic conditions (Sayer 2005). Carp is characterized by high tolerance for low oxygen concentration in water. It can administer oxygen efficiently in the period of hypoxia (or even anoxia) for example by accumulation of high amounts of glycogen in brain (Vornamen & Paajen 2006). Bowfin is a species which also can survive in a water with low oxygen level, but the mechanism is significantly different. It has a swim bladder transformed to lung what makes it possible to breath an air, when using gills is not sufficient (Daxboeck et al. 1981).We expect that bowfin should cope better with poor oxygen conditions because of that diversified possibilities.

It is known that hypoxia and hyperoxia affect carp's blood plasma activity of glutathione peroxidase, an enzyme protecting organism from oxidative damage, there was 37% increase of activity of this enzyme in hyperoxia and 38% decrease in hypoxia. The oxidative DNA damage was higher in both hypoxic and hyperoxic conditions. Also hematological parameters showed enhanced values under hypoxic conditions. The damage to liver and gills occurred due to both treatments. The specific growth rate was also affected in hypoxic conditions by 30% and was correlated with DNA damage. Prolonged hypoxic and hyperoxic conditions are responsible for oxidative stress responses at DNA and tissue levels (D'Aoust 1969, Mustafa et al. 2011). However until now experiments used only short term exposures (up to 30 days) and were performed on developed fish. There was no study attempting to check influence shortly after hatching for initial growth process which is crucial for organism. Moreover there has been no research of this kind for bowfin at all. While carp's growth rate decreases during low oxygen concentration (Mustafa et al. 2011) we want to compare it with the bowfin's growth rate to estimate the effectiveness of this two species growth in different oxygen conditions. The differences between species with distinctive oxygen intake could enhance our reasoning concerning oxygen scarcity as limiting factor for growth.

We are aware that differences from studying only two species may arise not solely from species response to studied factor but due to their species characteristics. It appears that study on wider range of species employing diverse oxygen intake are needed. Yet, because of the degree of technical complication it would be much more demanding. Our study could be preliminary for further experiments if the outcome would be satisfying.

3. *What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?*

In our experiment 300 carps and 300 bowfins will be distributed randomly into twenty four 100 l water tanks (25 fish of single species in each). This way each species and each treatment will be present in four tanks (Tab. 1). The methodology of achieving experimental oxygen conditions were followed after Mustafa et al. 2011. Normoxia (control group) at 7.1 ± 1.04 mg O_2 l^{-1}, hypoxia at 1.8 ± 1.1 mg O_2 l^{-1}will be achieved by pumping 99,99% nitrogen gas and hyperoxia at 12.3 ± 0.5 mg O_2 l^{-1}will be created by pumping oxygen of 99,95% purity. To keep temperature constant we will use water baths. Temperature and oxygen concentrations will be controlled constantly and each experimental box will have separate sensor connected to the gases suppliers. Water temperature will be maintained at 20°C and pH at 6,5-8,0 while photoperiod 12h light/12h dark. Fish with visible defects will be removed from the tanks, and if so the total volume in the tank shall be proportionally decreased to control for density. Experiment will last for six months and fish will be automatically fed with automatic feeders (*Naididae*, *Lumbricidae* worms and *Chironomidae* flies plus vitamin supplementation) every four hours to ensure unlimited food access for every individual. To eliminate all types of contamination (faeces, bacteria, food remains) active filtration (mechanical, biological and UV treatment) will be applied. Fish will be weighted every three days (all fish in one tank will be weighed and outcome divided by number of fish).

Fertilized bowfins' eggs will be purchased (there is no need for special permissions) from breeding lab at School of Environment and Natural Resources, Ohio State University (transport under permanent aeration) and the carps' fertilized eggs from local suppliers. During the first 21 days of life (until bowfin is fully capable of atmospheric oxygen usage (Ballard 1986, Dąbrowski, pers. comm.)) fish will be kept in aquariums with normoxic conditions and fed with *Artemia*. After experiment termination fish could be turned over to zoological gardens as animal fodder.

Tab. 1. A schematic illustration of breeding set of tanks. In each tank there will be 25 specimens

	Hypoxia		Normoxia		Hyperoxia	
Cyprinus carpio	HYP 1C	HYP 2C	NORM 1C	NORM 2C	HYPER 1C	HYPER 2C
	HYP 3C	HYP 4C	NORM 3C	NORM 4C	HYPER 3C	HYPER 4C
Amia calva	HYP 1A	HYP 2A	NORM 1A	NORM 2A	HYPER 1A	HYPER 2A
	HYP 3A	HYP 4A	NORM 3A	NORM 4A	HYPER 3A	HYPER 4A

Whether influence of treatment on growth rate (initial weight vs. final weight) is different between species (Fig. 1) shall be assessed by interaction. We will employ ANOVA with both factors fixed.
$GR=\mu+S+O+(SO)+e$
(GR-growth rate; S-species; O-oxygen conditions)

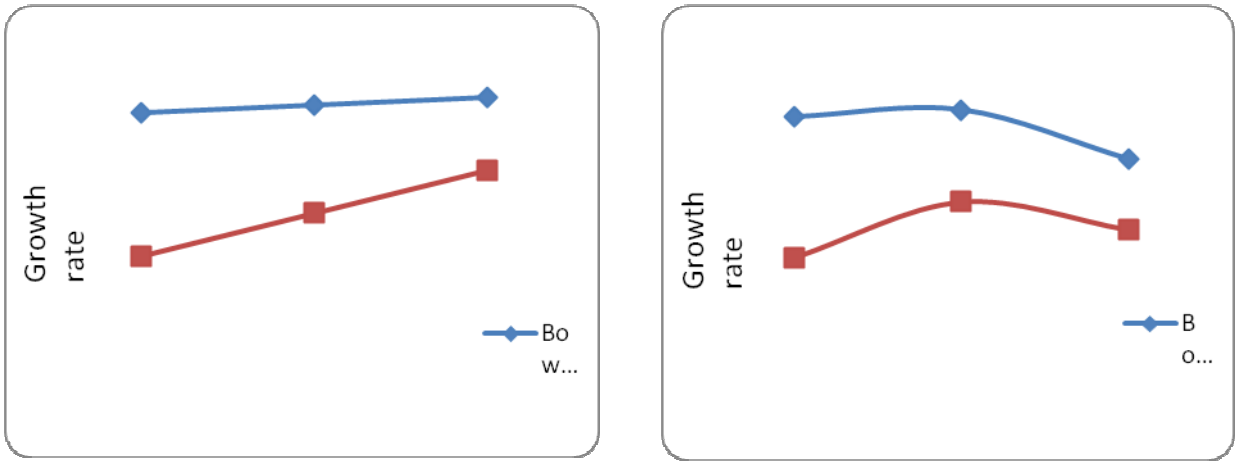

Fig. 1. Possible expected outcome of treatment on growth rate

To assess dynamic of weight gain growth curves will be created (on the basis of all measurements) and their parameters will be compared. After transforming the data repeated measure ANOVA will test for existing differences among curves.

4. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

   Our results may be beneficial for understanding role of limiting factor - oxygen concentration on crucial life-history trait which is growth dynamics. It may help avoiding errors in commercial fish farming and it may appear if bowfin's breeding would be possible and more economical in low oxygenated ponds. We plan to share our findings on international forums by means of articles in biological journals and participation in at least two conferences such as World Fisheries Conference, The International Congress on the Biology of Fish or Fisheries and Marine Sciences Conference.

References

Ballard W. W. 1986. Stages and rates of normal development in the holostean fish, Amia calva. J. Exp.Zool. 238 (3): 337–354.

D'Aoust B.G. 1969. Hyperbaric oxygen: toxicity to fish at pressures present in their swimbladders. Science 163 (3867): 576-578.

Daxboeck C., Barnard D.K., Randall D.J. 1981. Functional morphology of the gills of the bowfin, Amia calva L., with special reference to their significance during air exposure. Respir. Physiol. 43(3): 349-364.

Lushchak V.I., Bagnyukova T.V. 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol 144: 283–289.

Mustafa S.A., Al-Subiai S.N., Davies S.J., Jha A.N. 2011. Hypoxia-induced oxidative DNA damage links with higher level biological effects including specific growth rate in common carp, Cyprinus carpio L. Ecotoxicology 20 (6): 1455-1466.

Nikinmaa M. 2002. Oxygen-dependent cellular functions—why fishes and their aquatic environment are a prime choice of study. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133:1–16.

Sayer M.D.J. 2005. Adaptations of amphibious fish for surviving life out of water. Fish and Fishereies 6: 186–211.

Soitamo A.J., Rabergh C.M.I., Gassmann M., Sistonen L., Nikinmaa M. 2001. Characterization of a hypoxia-inducible factor (HIF-1a) from rainbow trout. Accumulation of protein occurs at normal venous oxygen tension. J. Biol. Chem. 276: 19699–19705.

van Raaij M.T.M., Breukel B.J., van den Thillart J.M., Addink A.D.F. 1994. Lipid metabolism of goldfish, (*Carassius auratus* L.).Hypoxia-induced oxidative DNA damage during normoxia and anoxia. Indications for fatty acid chain elongation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 107: 75–84.

Vornanen M., Paajanen V. 2006. Seasonal changes in glycogen content and Na+-K+-ATPase activity in the brain of crucian carp. Am. J. Physiol. Regul. Integr. Comp. Physiol. 291(5): 1482-1489.

Wu R.S.S. 2002. Hypoxia: from molecular responses to ecosystem responses. Mar. Pollut. Bull. 45: 35–45.

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yy)	Expected cost (zł)
1	The purchase of equipment	04.2013	40710
2	The purchase of fish	05.2013	6900
3	Fish breeding	12.2013	25983
4	Data processing	03.2014	8200
5	Preparation of papers and conference participation	09.2014	10000
		Total	91793

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)		
		2013	2014	Total
1	Direct costs, including:	56610	14000	70610
	1/ Salaries and benefits	9000	4000	13000
	2/ Equipment	0	0	0
	3/ Other direct costs	47610	10000	57610
2	Indirect costs	16983	4200	21183
3	Total costs (1+2)	73593	18200	91793

Details of direct cost items

1/Salaries and benefits
Project managers salary : 10000 zł
Technical staff salary: 3000 zł
3/ Other direct costs
24 100 l fish tanks: 3500 zł
24 water baths: 6000 zł
24 automatic feeders, 3600 zł
24 filters with UV lamp, 6000 zł
24 temperature sensors: 2500 zł
24 oxygen concentration sensors: 5000 zł
2 oxygen cylinders + refilling: 3000 zł
2 nitrogen cylinder s+ refilling: 5000 zł
weight: 200 zł
1 pH meter 100 zł
350 bowfins: 1750 zł
300 carps: 150 zł
fish food and vitamin supplements: 5000 zł
bowfin's transportation: 5000 zł
10 plastic containers for fish weighting: 50 zł
net: 10 zł
hygienic materials (gloves, paper towels, protective clothing): 250zł
books and journals: 500 zł
conferences (for 2 people): 10000 zł

4.2. Influence of dietary restriction on life strategy of household cricket (Acheta domesticus)

Authors: Justyna Gutowska, Diana Maciąga

4.2.1. First version of the project

Project summary (not to exceed 1 page; may be published by KBN if grant is awarded)

Reproduction has been confirmed as highly expensive in terms of energy use. However, it is maximized by natural selection at the cost of other energetically expensive life history features such as lifespan prolongation. Though govern by energetically expensive mechanisms, lifespan prolongation is a common effect of limited availability of energy. Consequently it should be accompanied by reduction in the amount of energy allocated in reproduction. We expect that the life strategy of household cricket (*Acheta domesticus*) subjected to dietary restriction will be altered in terms of lifetime output of offspring.

In order to answer this question we assess the relation between lifetime fecundity and lifespan in crickets under different dietary regimes. Two groups of mature female crickets will be subjected to different dietary regimes: unlimited food access vs dietary restriction. Lifespan of each individual will be recorded as well as lifetime fecundity, to which two different approach will be applied: fecundity understood as total number of eggs or as dry mass of eggs laid throughout females' life.

We predict that if there is a trade-off between energy allocation in the two above mentioned energetically expensive life history features, the dietary restriction should result in prolonged lifespan accompanied with reduction of lifetime fecundity regardless of the approach.

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

1. What problem is being proposed and why?

According to the theory of evolution, reproduction resulting in propagating genes in future gene pool is animals' predominant reason for existence. However, producing offspring is highly

energy consuming, thus it has been shaped by natural selection to maximize the lifetime reproductive output of an individual. In favorable conditions the lifetime reproductive success is maximized by allocating resources in breeding at the cost of mechanisms enforcing longevity, which maintenance is energetically expensive. In times of energy shortage, the survival of an individual requires allocating available resources to vital functions of an organism, which might result in restricting other energy consuming activities, such as breeding. It has been clearly showed, however, that lower availability of energy in the form of dietary restriction results in increasing lifespan in both, vertebrates and invertebrates, which in turn should enable them to survive the period of unfavorable conditions and proceed to breeding (Spearman 2011). The significance of the relation between prolonging lifespan and changes in fecundity of mature insects remains open to debate.

In the proposed experiment, we will test whether dietary restriction alters life strategy of mature females of household cricket (*Acheta domesticus*).

We expect that dietary restriction should result in prolonged lifespan accompanied with reduction of lifetime fecundity understood as both, total number of eggs and dry mass of eggs laid throughout females' life.

2. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

The biological processes occurring during lifetime that are referred to as aging and their evolutionary significance has been widely investigated in vertebrates (Spearman 2011). However, some of their features, such as their longevity, size, ethical restriction and expense can constrain their use as model organisms. Invertebrates can thus be more suitable models for studying the mechanisms that can slow aging. *Drosophila melanogaster* is an especially widely used insect model. While fruit flies are a superb experimental system for gene manipulation, they are too small for experiments that involve tracking or observing individuals throughout adult life or egg collecting. Household cricket has been recently proposed as an alternative model organism for aging related studies (Lyn et al. 2011).

Aging processes have strong genetic basis. Diet is the only environmental variable that has been shown to affect the rate of aging in a wide range of species (Fuller L. E. 2007). Dietary restriction (intermittent access to food) has already been assessed as a suitable method of

provoking longevity prolongation as intermittent feeding of crickets significantly reduces overall intake despite compensation of food intake that tend to occur during periods of food availability (Lyn et al. 2011).

Dietary restriction often acts in part by delaying or reducing allocation to reproduction, through a trade-offs between early fecundity and longevity (Hatle et al. 2006). It has been proved that dietary restriction (DR) delays maturation age, slows down juvenile growth and results in lower body size and mass upon maturation (Lyn et al. 2011). As those are critical and inter-related life history features, resource shortfalls increase risks of mortality and reproductive failure (Lyn et al. 2011). Immature insects are also more sensitive to inadequate diets than adults, what increases early mortality (Lyn et al. 2011). By choosing only mature crickets bred on unlimited food access for the experiment we exclude the factor of delaying reproduction. This will also prevent food dependent differences in body mass and size upon maturing age that are proved to affect egg size and number (Olcay 2008) and reduce the risk of increased mortality. It has been also confirmed in several studies (Jura 1988) that the size, to which the crickets grow, is genetically coded: they grow up to reaching adequate size. Therefore, there should be no effect of diet restrictions on growth in crickets' adulthood and the diet restriction is the only factor that can influence fecundity.

In most studies on DR influence on ageing with *D. melanogaster*, used as model organism, adult individuals are subjected to treatment. However, the research concerning impact of DR on lifespan in which *A. domesticus* is used were restricted strictly to DR applied to young or juvenile individuals. Thus the relation between lifespan and fecundity has not been yet investigated in case of mature crickets (Lyn et al. 2011) and remains open to debate.

The research conducted by Lyn et al. (Lyn et al. 2011) also revealed gender specific differences in the impact of DR on cricket life cycles. Unlike females, in case of males the increase in longevity was entirely due to delayed maturation, the factor we have excluded from our study. Consequently we have decided on using female crickets as model organisms.

In our study we subject adult females of *A. domesticus* to dietary restriction. We expect that the life strategy of household cricket subjected to dietary restriction will be altered in terms of lifetime output of offspring. Dietary restriction should result in prolonged lifespan accompanied with reduction of lifetime fecundity.

3. *What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?*

a) model organism

Household crickets (*Acheta domesticus*) will be used as model organisms. *A. domesticus* is ideal for such study since it is omnivorous and lives only approximately 120 days at 30°C (Lyn et al. 2011). Nymphs and adults have similar requirements. Large cohorts of known age can be generated from eggs, growth is easily monitored and gender can be discerned at young ages. Maturation is easily detected by expression of wings and adult genitalia (Lyn et al. 2011)

b) breeding protocol

The purchased colony will consist of 20 crickets. The individuals will be housed in an aquarium (100 x 40 x 50cm) with egg carton shelters. The top will be covered with 1 mm^2 plastic mesh that prevent escape while providing ventilation. Holes (1 mm) in the lids will provide ventilation. Chicken feed, fresh carrots and dechlorinated (boiled) water will be provided ad libitum. The nesting material will be sprayed with dechlorinated water daily. The colony will be maintained at 30°C with a 12 h light/12 h dark photoperiod.

Crickets' natural nesting material is damp soil and so to duplicate this a plastic boxe full of moist nesting material (coco peat, successfully used while breeding crickets) will be placed on top of the egg cartons in the breeding container. The nesting boxes full of eggs will be removed and incubated in smaller boxes (23,5 x 16,5 x 12cm).

c) experiment protocol

60 females and 60 males of the same age will be randomly chosen for the experiment. Upon maturity, the crickets will be randomly divided in two groups. Animals assigned to the dietary restricted group (DRG) will have access to food for only 12 hours separated by 24 h intervals. Water will be available ad libitum. Control group (CG) will have constant access to food and water ad libitum. Each of two groups will consist of 30 females and 30 males.

The individuals will be housed in plastic enclosures (23,5 x 16,5 x 12cm) with egg carton shelters: each female with one randomly chosen male. Shallow trays filled with coco peat will be provided in each enclosure as a place for oviposition. In DR group, the male will be removed from

the box for the duration of female's fast, so that the male diet remains unchanged. In control group the male will also be removed from the box for 24 h intervals.

Every three days the medium containing eggs will be removed, and replaced with fresh egg substrate pod. The eggs will be then extracted and counted, dried and weighted. Total number of eggs, total dry mass of eggs and lifespan will be recorded for each female.

d) statistical analysis

To exclude the possibility that smaller number of eggs may be compensated by allocation of resources resulting in their bigger size, we will carry out analysis for two approaches: in first lifetime fecundity will be understood as the number of eggs laid by female during lifetime, in second - by the dry mass of the total number of eggs per female.

To determine whether the groups differ in the observed relation between lifetime fecundity and lifespan, analysis of covariance with special interest in interaction between lifespan and fecundity will be applied. However, we are aware that the dietary restriction affects lifespan and so this variables cannot be treated as independent. To avoid this, regression will be used separately for both groups as an alternative, then followed by the test for differences in slopes. This will be applied for both approaches – number of eggs and dry mass of eggs. We predict that the obtained results should match the following graph:

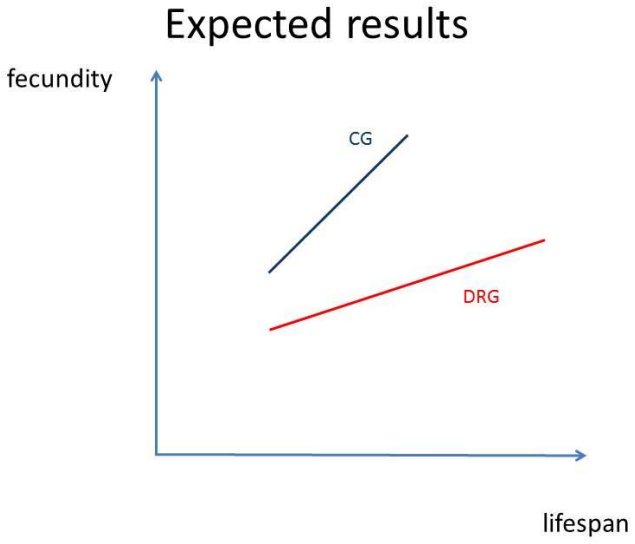

Fig. 1. Expected results of the experiment. DRG – dietary restriction, CG – control.

4. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

The results will contribute to the knowledge of life strategy trade-offs related to dietary restriction. They will also supplement research on lifespan prolongation with *A. domesticus* as a model organism as no data for dietary restriction influence on life history features tested on mature individuals is available.

We plan to present our results at two conferences on international and national scale. We also intend to publish two papers in refereed scientific journal such as *AGE: Journal of the American Aging Association* or *Mechanisms of Aging and Development.*

References

Fuller L. E., 2007. Age and Diet Strongly Affect Total Fat Body Protein in Lubber Grasshopper. University of North Florida Digital Commons. All Volumes (2001-2008)

Hatle J. D., Wells S. M., Fuller E., Allen I. C., Gordy L. J., Melnyk S., Quattrochi J., 2006. Calorie restriction and late-onset calorie restriction extend lifespan but do not alter protein storage in female grasshoppers. Mechanisms of Ageing and Development 127: 883-891

Jura C. 1988. Biologia rozwoju owadów – Państwowe Wydawnictwo Naukowe, Warszawa (4).

Lyn J. C., Naikkhwah W., Aksenov V., Rolli C. D., 2011. Influence of two methods of dietary restriction on life history features and aging of the cricket Acheta domesticus. AGE 33: 509-522

Olcay A., Whitman D, 2008. Analysis of body size and fecundity in a grasshopper. Journal of Orthoptera Research 17: 249-257

Spearman J. R., Mitchell S. E., 2011. Molecular Aspects of Medicine. Special Issue. Caloric Restriction 32: 159-22I

SOLICITED PROJECTS ONLY: DOES THE APPLICANT MEET THE CALL CRITERIA, PARTICULARLY THOSE CONCERNING INTERNATIONAL COOPERATION?

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yyyy)	Expected cost (zł)
1	Laboratory preparation – purchasing necessary equipment and materials	01/2013	4 600
2	Breeding purchased colony for selection of crickets in the same age	02/2013	1 528
3	Experimental breeding and data collection	08/2013	18 168
4	Analysis of the results	09/2013	3 000
5	Popularisation of the results - preparation of the manuscript for publication, participation in the international conference	1.12.2014	12 000
		Total	39 296

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)			
		2013	2014	2015	Total
1	Direct costs, including:	39 296	-	-	39 296
	1/ Salaries and benefits	30 000	-	-	30 000
	2/ Equipment	-	-	-	-
	3/ Other direct costs	9 296	-	-	9 296
2	Indirect costs	11 789	-	-	11 789
3	Total costs (1+2)	51 085	-	-	51 085

Details of direct cost items

1/ Salaries and benefits

- 2 investigators – 20 person-months, 30 000zł

2/ Equipment

- (all the necessary equipment such as scales and drier are accessible at our University)

3/ Other direct costs

- 20 alive crickets – 20zł
- Aquarium for the purchased colony breeding – 250zł
- Aquarium lamp – 100zł
- Enclosures for the experimental breeding – 2 050zł
- Coco peat 350 litters – 112 zł
- Chicken food and fresh carrots for feeding the crickets – 596zł
- Nesting boxes (60 small boxes and 1 big for the original colony) – 65zł
- Food and water dishes (260) – 52zł
- Egg cartons – 50zł
- Conference attendance (plane ticket, daily allowance, conference fee) for 1 person – 6 000zł

4.2.2. Reviews

- Adam Łomnicki

Review of the grant proposal:
"Influence of dietary restriction on life strategy of household cricket (Acheta domesticus)"

All the elements required in the grant proposal are given here, but the author exhibit fundamental misunderstanding what is the aim the scientific investigations. The science is not to carry on similar studies like others which were already published, with small modifications only (different species, different age of animals used in the experiment), in order to be sure we get the expected results. Science is made for finding something quite new and unexpected, something that will change our view of the World and will introduce changes in the textbooks. This proposal is not ambitious. It repeats well-known experiments with very small modifications. There is no clearly and univocally stated hypothesis of hypotheses with their alternatives.

Many researchers after completing the project find that the results are not very interesting but anyhow they publish it in order to bring about some small bits of information to others. This is normal situation. But when one starts the project and writes a grant proposal, this proposal should looks like its results being published in Nature or Science. If it looks like being hardly published in "Zeszyty Naukowe Uniwersytetu Jagiellonskiego", it is better to do other job, not science.

The proposal should have clearly stated hypotheses with outcome of the experiment tied with each hypothesis and the important consequences of accepting this or other

hypothesis. The consequences should be of importance either for the theory of biology or for practical applications. Without it the proposal in dull and of little interest.

Other parts of the proposal: the methods, the equipment required, and the budget look like being well done. However if there are some mistakes made in these parts, they can be justified since the authors have no access to the resources available in the Institute and in the city of Krakow. But there is no excuse for the shortage of knowledge and imagination which would allow to produce a. ground breaking proposal.

- Beata Klimek

Review of Project: Influence of dietary restriction on life strategy of household cricket (Acheta domesticus).

Proposed research project has on the aim assessing the trade-off for energy allocation between animal fecundity and lifespan, depending on dietary restrictions. Authors assume, that dietary restrictions will result in prolonged lifespan accompanied with reduction of lifetime fecundity.

The problem chosen is interesting and ambitious. However, it could be difficult to verify this hypothesis in proposed experiment. My main doubtful concern to measure of fecundity. I am not sure, if just number of eggs and even their dry mass is a sufficient measure of female fecundity for the project purpose. I suggest to complete the experiment with eggs hatchability tests and, maybe, eggs calorimetric analysis and fat/protein content.

Household cricket seems to be ideal test species, as described in part 2: Present state of knowledge (why not in part 3: Proposed methodology?). However, authors should be cautious with planning dietary restriction experiments with cricket. There was showed, that this species shows cannibalism in case lack of valuable food. Starving female may consume male during the visit as well as laid eggs. Proposed solution of this hitch has to be described or authors should consider use another species.

Budget of project is adequate. In my opinion, authors will need more time to realize the project than planned one year. There can occur problems with animals breeding (for example, unexpected high mortality), lack of available laboratory space or any other possible disturbance. Appropriate conference may take place even a half year later after abstract submission. Also publishing the paper, including its preparation may take much longer than planned few weeks, causing problems with project final statement.

Other remarks:

Summary contains necessary information, but relatively short. Authors can add two or even three sentences more and present project proposal more comprehensively.

(Spearman 2011) should be cited in the text as (Spearman and Mitchell 2011). Also, (Olcay 2008) should be given as (Olcay and Whitman 2008).

Between expected project results, there is not given PhD thesis.

First task in the project schedule (purchasing equipment etc.) is not a research task and should be removed.

Summing up, I believe that after few small corrections, the project will be successful.

- Ewa Chmielowska

Dear Researchers,

The presented project is carefully written and fulfills presented requirements. It consists of all necessary parts, presents clearly the research problem, and presents a coherent logical model for hypothesis testing. However, there are some questions I would like to raise.

1 Generally, many sentences are too long. (It is my problem, too). This is not the right place to use complicated syntax. The shorter the sentence, the clearer its meaning is to re reader. For example:

In times of energy shortage, the survival of an individual requires allocating available resources to vital functions of an organism, which(here you may start a new sentence: It) might result in restricting other energy consuming activities, such as breeding.

2 In my opinion, the first paragraph of abstract may be written more concise.

3 I am not convinced, that Drosophila melanogaster is too small for experiments tracking individuals throughout adult life or egg collecting. We can even observe courtship patterns within single individuals in this species, and even smaller organisms like Caenorhabditis elegans are used in similar studies on life history. I need better explanation.

4 Is this a novel research, or you are following already existing results?

5 Will you choose the crickets of the same size, if it is related to its fecundity?

6 Very minute problem: no coma in the sentence:

However, the research concerning impact of DR on lifespan in which A. domesticus is used, were restricted strictly to DR applied to young or juvenile individuals.

Unlike females, in case of males, the increase in longevity was entirely due to delayed maturation

7 You are basing merely on one paper, reviewing the current state of knowledge in this field, maybe one is not enough? (lets assume it is because of our time limitation)

8 Will housing together not affect the experiment results? It is possible that airborne molecules, such as pheromones will affect the whole group? Will there be any food competition?

9 How will you control male effect on egg size (if such mechanism in crickets exists at all)?

10 Tap water chemical composition vary in each region- if you want your experiment fully reproductible- you should control this variable, too.

11 There is no information whether and why you are skilled enough to perform this experiment

12 When will you stop the experiment? After all subjects die? How will you manage with incomplete data within survivors?

Generally, it is reasonable to work with language form of this project, but proposed question and experimental model seem well developed.

- Damian Kolbe

Review of project: „Influence of dietary restriction on life strategy of household cricket"

The topic of the proposal is clean and present to us very interesting type of physiologic experiment, but I doubt it's need to write MA degree in applicants information. This project show us how can we linked good idea, not difficult methodology and low cost budget. Project included all necessary parts: abstract, project description, methodology, expected results. It's good that applicant choose mature females of cricket as a model organism, because they are good material too observation, easy to breeding and don't need too much space in laboratory. In formal side paper is written without language mistakes.

It's good idea that to exclude the possibility of difference eggs size the applicants compensated this in dry mass of eggs. Suggested statistical methods looks appropriate for analyzing the data. Expected results are reasonable. All references appears in the text.

I find some mistakes in budget construction: aquarium, aquarium lamp, nesting boxes, and rest of the experimental equipment should be belong to equipment in "details of direct cost item". Moreover as I write earlier the presented grant proposals is really well prepared and give big chance to get succeed.

- Michał Kuciel

Revision of the project entitled: 'Influence of dietary restriction on life strategy of household cricket (Acheta domesticus)'.

In proposed for review project Authors want to find out if dietary restriction will result in prolonged lifespan accompanied with reduction of lifetime fecundity.

The hypothesis is interesting and placed correctly. Predictions are clearly defined.

Abstract gives every needed information without excess.

Lack of keywords.

The Authors gave a well grounded the aim, relevance of the problem and the choice of species for the experiment.

Proposed budget:

Those positions from 'Other direct costs' should be in the 'Equipment':

Aquarium for the purchased colony breeding,

Aquarium lamp

Enclosures for the experimental breeding

Nesting boxes (60 small boxes and 1 big for the original colony)

- Joanna Sudyka

Review of the grant proposal: Influence of dietary restriction on life strategy of household cricket (Acheta domesticus)

The project appears as well thought and neat, research problem is accurately outlined. It may also be innovative even though caloric restriction has been extensively studied. It is comprehensible and easy to read.

Abstract is quite clear, the expectations are stated, but there are no alternative biological hypotheses mentioned. The approach proposed by the authors – fecundity assessed by the total number of eggs and as dry mass is quite original. However there is no clear remark on why the proposed experiment is essential to conduct, how is it different from previous ones. State of knowledge is described sufficiently for understanding the study issue. In some places I have an impression that there are repetitions of the same thoughts or densely packed citations of the same source (ex. Lyn et al. 2011) that do not bring anything new to the project.

Some technical problems need more attention. Aquariums, enclosures and nesting boxes should be rather placed under equipment and not other costs. I have noticed some minor mistakes like not removed spare columns in budget table, dot after title or incoherent

punctuation in the text and references which require second look. There are also some grammatical ambiguities which make few sentences a bit awkward (ex. line 45, 51, 71).

The project does not have many weak points, good points such as graphical presentation of expected results prevail and is definitely worth undertaking after better justification for its realization.

4.2.3. Final version of the project

Project summary

Reproduction has been confirmed as highly expensive in terms of energy use. However, it is maximized by natural selection at the cost of other energetically expensive life history features such as lifespan prolongation which is a common effect of limited availability of energy despite being governed by energetically expensive mechanisms. Consequently it should be accompanied by reduction in the amount of energy allocated in reproduction. Thus we expect that the life strategy of household cricket (*Acheta domesticus* Linnaeus, 1758) subjected to dietary restriction will be altered in terms of lifetime output of offspring. We want to get to know what is this trade-off between prolonged lifespan and fecundity.

In order to answer this question we assess the relation between lifetime fecundity and lifespan in crickets under different dietary regimes. Two groups of mature female crickets will be subjected to different dietary regimes: unlimited food access vs dietary restriction (DR). Lifespan of each individual will be recorded as well as lifetime fecundity, to which two different approaches will be applied: fecundity understood as total number of eggs or as dry mass of eggs laid throughout females' life.

We predict that if there is a trade-off between energy allocation in the two above mentioned energetically expensive life history features, the dietary restriction should result in prolonged lifespan accompanied with reduction of lifetime fecundity understood as both, total number of eggs and total mass of eggs laid throughout females' life.

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

1. *What problem is being proposed and why?*

According to the theory of evolution, reproduction resulting in propagating genes in future gene pool is animals' predominant reason for existence. However, producing offspring is highly energy consuming, thus it has been shaped by natural selection to maximize the lifetime

reproductive output of an individual. In favorable conditions the lifetime reproductive success is maximized by allocating resources in breeding at the cost of mechanisms enforcing longevity, which maintenance is energetically expensive. In times of energy shortage, the survival of an individual requires allocating available resources to vital functions of an organism, which might result in restricting other energy consuming activities, such as breeding. It has been clearly showed, however, that lower availability of energy in the form of dietary restriction results in increasing lifespan in both, vertebrates and invertebrates, which in turn should enable them to survive the period of unfavorable conditions and proceed to breeding (Spearman 2011). The significance of the relation between prolonging lifespan and changes in fecundity of mature insects remains open to debate.

In the proposed experiment, we will test how dietary restriction alters life strategy of mature females of household cricket (*Acheta domesticus* Linnaeus, 1758). We ask if the energy shortage causes number of eggs decrease or e.g. total mass of an egg decrease. We expect that also both at the same time are possible.

2. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

The biological processes occurring during lifetime that are referred to as aging and their evolutionary significance has been widely investigated in vertebrates (Spearman 2011). However, some of their features, such as their longevity, size, ethical restriction and expense can constrain their use as model organisms. Invertebrates can thus be more suitable models for studying the mechanisms that can slow aging. *Drosophila melanogaster* is an especially widely used insect model. While fruit flies are a superb experimental system for gene manipulation, their small size impedes the experiments that involve tracking or observing individuals throughout adult life or egg collecting. Household cricket has been recently proposed as an alternative model organism for aging related studies (Lyn et al. 2011).

Aging processes have strong genetic basis. Diet is the only environmental variable that has been shown to affect the rate of aging in a wide range of species (Fuller L. E. 2007). Dietary restriction (intermittent access to food) has already been assessed as a suitable method of provoking longevity prolongation as intermittent feeding of crickets significantly reduces overall

intake despite compensation of food intake that tend to occur during periods of food availability (Lyn et al. 2011).

Dietary restriction often acts in part by delaying or reducing allocation to reproduction, through a trade-offs between early fecundity and longevity (Hatle et al. 2006). It has been proved that dietary restriction (DR) delays maturation age, slows down juvenile growth and results in lower body size and mass upon maturation (Lyn et al. 2011). As those are critical and inter-related life history features, resource shortfalls increase risks of mortality and reproductive failure (Lyn et al. 2011). Immature insects are also more sensitive to inadequate diets than adults, what increases early mortality (Lyn et al. 2011). By choosing only mature crickets bred on unlimited food access for the experiment we exclude the factor of delaying reproduction. This will also prevent food dependent differences in body mass and size upon maturing age that are proved to affect egg size and number (Olcay 2008) and reduce the risk of increased mortality. It has been also confirmed in several studies (Jura 1988) that the size, to which the crickets grow, is genetically coded: they grow up to reaching adequate size. Therefore, there should be no effect of diet restrictions on growth in crickets' adulthood and the diet restriction is the only factor that can influence fecundity.

In most studies on DR influence on ageing with *D. melanogaster*, used as model organism, adult individuals are subjected to treatment. However, the research concerning impact of DR on lifespan in which *A. domesticus* is used were restricted strictly to DR applied to young or juvenile individuals. Thus the relation between lifespan and fecundity in case of mature crickets (Lyn et al. 2011) remains open to debate.

The research conducted by Lyn (Lyn et al. 2011) also revealed gender specific differences in the impact of DR on cricket life cycles. Unlike females, in case of males the increase in longevity was entirely due to delayed maturation, the factor we have excluded from our study. Consequently we have decided on using female crickets as model organisms.

In our study we subject adult females of *A. domesticus* to dietary restriction. We expect that the life strategy of household cricket subjected to dietary restriction will be altered in terms of lifetime output of offspring. Dietary restriction should result in prolonged lifespan accompanied with reduction of lifetime fecundity.

3. *What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?*

a) model organism

Household crickets (*Acheta domesticus*) will be used as model organisms. *A. domesticus* is ideal for such study since it is omnivorous and lives only approximately 120 days at 30°C (Lyn et al. 2011). Nymphs and adults have similar requirements. Large cohorts of known age can be generated from eggs, growth is easily monitored and gender can be discerned at young ages. Maturation is easily detected by expression of wings and adult genitalia (Lyn et al. 2011).

b) breeding protocol

The purchased colony will consist of 20 crickets. The individuals will be housed in an aquarium (100cm x 40cm x 50cm) with egg carton shelters. The top will be covered with 1 mm^2 plastic mesh that prevent escape while providing ventilation. Holes (1 mm) in the lids will provide ventilation. Chicken feed, fresh carrots and dechlorinated (boiled) water will be provided ad libitum. The nesting material will be sprayed with dechlorinated water daily. The colony will be maintained at 30°C with a 12 h light/12 h dark photoperiod.

Crickets' natural nesting material is damp soil and so to duplicate this a plastic boxe full of moist nesting material (coco peat, successfully used while breeding crickets) will be placed on top of the egg cartons in the breeding container. The nesting boxes full of eggs will be removed and incubated in smaller boxes (23,5cm x 16,5cm x 12cm). Mesh with holes enough big for ovipositor will cover nesting material, to enable oviposition and prevent digging the eggs by adult crickets.

c) experiment protocol

60 females of the same age will be randomly chosen for the experiment. (Alternatively, individuals of similar size will be chosen to avoid female size effect on fecundity). Upon maturity, female crickets will be randomly divided in two groups. Females assigned to the dietary restricted group (DRG) will have access to food for only 12 hours separated by 24 h intervals. Water will be available ad libitum. Control group (CG) will have constant access to food and water ad libitum. Each of two groups will consist of 30 females.

The individuals will be housed in plastic enclosures (23,5 x 16,5 x 12cm) with egg carton shelters: during the first week of dietary restrictions each individual in one box and after that each female with one randomly chosen male. Shallow trays filled with coco peat will be provided in

each enclosure as a place for oviposition. We will put screen on the surface of the soil to prevent crickets from digging or eating the eggs. In both groups, the male will be removed from the box for the duration of female's fast, so that the male diet remains unchanged and conditions for the two groups of females remain the same. Each time the male will be removed, it will be placed in random female's box to reduce possible effect of male on fecundity.

Cohorts of 70 males will be randomly chosen for the experiment. Bigger number of males than females is just to assure each female will be able to be accompanied by a male in case some of males will unexpectedly die before they are supposed to stop taking part in the experiment. After each 60 days we will change the cohort of males for the new, younger one as they are expected to die earlier than females from DR group (all males have unlimited access to food) and we want to assure having randomly chosen males till the end of experiment (experiment will end with death of the last female).

Every three days the medium containing eggs will be removed, and replaced with fresh egg substrate pod. The eggs will be then extracted and counted, dried and weighted. Total number of eggs, total dry mass of eggs and lifespan will be recorded for each female.

d) statistical analysis

To exclude the possibility that smaller number of eggs may be compensated by allocation of resources resulting in their bigger size, we will carry out analysis for two approaches: in first lifetime fecundity will be understood as the number of eggs laid by female during lifetime, in second - by the dry mass of the total number of eggs per female.

To determine whether the groups differ in the observed relation between lifetime fecundity and lifespan, analysis of covariance with special interest in interaction between lifespan and fecundity will be applied. However, we are aware that the dietary restriction affects lifespan and so this variables cannot be treated as independent. To avoid this, regression will be used separately for both groups as an alternative, then followed by the test for differences in slopes. This will be applied for both approaches – number of eggs and dry mass of eggs. We predict that the obtained results should match the following graph:

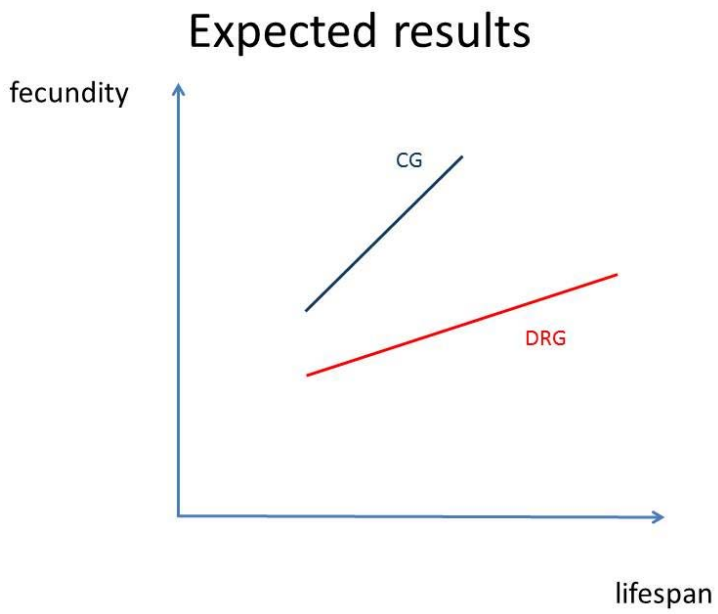

Fig. 1. Expected results of the experiment. DRG – dietary restriction, CG – control.

4. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

The results will contribute to the knowledge of life strategy trade-offs related to dietary restriction. They will also supplement research on lifespan prolongation with *A. domesticus* as a model organism as no data for dietary restriction influence on life history features tested on mature individuals is available.

We plan to present our results at the conference on international scale. We also intend to publish two papers in refereed scientific journal such as *AGE: Journal of the American Aging Association* or *Mechanisms of Aging and Development.*

References

Fuller L. E., 2007. Age and Diet Strongly Affect Total Fat Body Protein in Lubber Grasshopper. University of North Florida Digital Commons. All Volumes (2001-2008)

Hatle J. D., Wells S. M., Fuller E., Allen I. C., Gordy L. J., Melnyk S., Quattrochi J., 2006. Calorie restriction and late-onset calorie restriction extend lifespan but do not alter protein storage in female grasshoppers. Mechanisms of Ageing and Development 127: 883-891

Jura C. 1988. Biologia rozwoju owadów – Państwowe Wydawnictwo Naukowe, Warszawa (4).

Lyn J. C., Naikkhwah W., Aksenov V., Rolli C. D., 2011. Influence of two methods of dietary restriction on life history features and aging of the cricket Acheta domesticus. AGE 33: 509-522

Olcay A., Whitman D, 2008. Analysis of body size and fecundity in a grasshopper. Journal of Orthoptera Research 17: 249-257

Spearman J. R., Mitchell S. E., 2011. Molecular Aspects of Medicine. Special Issue. Caloric Restriction 32: 159-22I

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yyyy)	Expected cost (zł)
1	Breeding purchased colony for selection of crickets in the same age	02/2013	7 967
2	Experimental breeding and data collection	08/2013	23 618
3	Analysis of the results	12/2013	7 800
4	Popularization of the results - preparation of the manuscript for publication, participation in the international conferences	12/2015	15 600
		Total	54 985

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)		
		2013	2014	Total

1	Direct costs, including:	30 296	12 000	39 296
	1/ Salaries and benefits	27 000	6 000	33 000
	2/ Equipment	-	-	-
	3/ Other direct costs	3 296	6 000	9 296
2	Indirect costs	9 089	3 600	11 789
3	Total costs (1+2)	39 385	15 600	54 985

Details of direct cost items

1/ Salaries and benefits

- 2 investigators – 20 person-months, 30 000zł

2/ Equipment

- (all the necessary equipment such as scales and drier are accessible at our University)

3/ Other direct costs

- 20 alive crickets – 20zł
- Aquarium for the purchased colony breeding – 250zł
- Aquarium lamp – 100zł
- Enclosures for the experimental breeding – 2 050zł
- Coco peat 350 litters – 112 zł
- Chicken food and fresh carrots for feeding the crickets – 596zł
- Nesting boxes (60 small boxes and 1 big for the original colony) – 65zł
- Food and water dishes (260) – 52zł
- Egg cartons – 50zł
- Conference attendance (plane ticket, daily allowance, conference fee) for 1 person – 6 000zł

4.3. The composition of urine influence male rat's sexual attractiveness

Authors: Ewa Chmielowska, Damian Kolbe

4.3.1. First version of the project

Project summary
Pheromone play important role in mammal communication. A little has been known about sexual pheromones in rat, despite it is one of the most important model animals in biology. This study aims to determine, if olfactory active compounds of male rat's urine are responsible for its sexual attractiveness. The bioassay with 80 sex-naive female Wistar inbreed rats in estrus phase will be conducted under controlled conditions in Y-maze. The females will divided into 4 groups and exposed to two samples of urine: One without any intervention, another one without (test 1- control) or with additional compound: squalene (test 2), 2-heptanone (test 3), 4-ethyl phenol (test 4). The basic urine sample will comprise of void urine samples of 5 male rats. The preference for one of two samples will be measured as a time the female subject spent in sample proximity. The deviation in urine sample preferences will be tested with t-test, and different attractiveness levels will be tested with Friedman test. It is expected, that some of the chemosignal urine compound may increase the attractiveness of male rat's urine, however it may occur, that the crucial determinant may be the proportion, not the concentration itself, and therefore increasing the level of one compound may have adverse effect of attractiveness

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

5. What problem is being proposed and why?

Many mammals use volatile molecules, the pheromones, to affect the behavior of other specimens they share the living space with. Pheromones carry the information about species, sex, health status, social position and current stress condition. Pheromones may consist of single compound, but may be also a complex of different molecules. As volatile molecules, the pheromones are very difficult to investigate, especially in quantitative way. Thus, it is difficult to assess the efficacy of single compounds of conjunct pheromones. Such situation occurs in rats, where at least 3 molecules were recently identified as sex pheromones.

The key question to answer in this research is whether the different proportions of 3 pheromone compounds in the urine may have an impact on attractiveness of a male rat.

Keywords
rat, pheromone, squalene, 2-heptanone, 4-ethyl phenol, sexual attractiveness

6. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

Despite the rat is one of major model animals for biological research, and has been even domesticated for this purpose, a little is known about chemosignals in this species. Researchers identified some pheromones in rats, including dodecyl propionate, a compound of rat pups preputial gland, that initiates
The voided urine and preputial gland secretion of the rat can convey a information
about species, sex, reproductive condition, and stress status, however, little is known about which compounds are involved in coding for the information recently.
Three compounds were identified as sex pheromones in male rats: squalene, 2-heptanone, and 4-ethyl phenol. These were identified by a bioassay, as it could restore an attractiveness of castrated rats' urine to sex-naive females. Adding any of the 3 compounds separately (at a concentration higher than its physiological level in male urine) to castrated male urine (CMU) increased the attractiveness of CMU to females, as well. However, adding the 3 together (at the levels in normal male urine) to CMU significantly increased the attractiveness of CMU to females. Nevertheless, such combination did not fully restore females' preference for urine from intact males, suggesting that some other trace compounds might also play some roles in sex attractiveness.

7. *What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?*

Experiment design and data processing

To test the hypothesis, the bioassay of urine samples will be carried.
There will be 4 assays conducted. For each assay, the group of 20 receptive (in estrus) female rats will be the placed in one branch of Y-maze and left to freely explore the maze for 2 minutes. In each of other two branches, there will be a sample of male rat urine, and the sample of urine without (test 1- control) or with additional compound: squalene (test 2), 2-heptanone (test 3), 4-ethyl phenol (test 4).

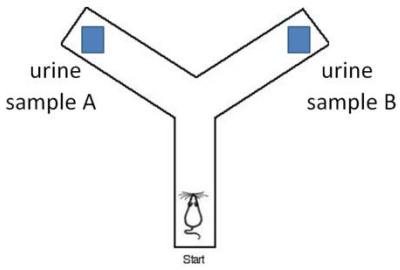

Fig.1. Y-maze

The female rat preference to the particular urine-pheromone mixture was expressed by the time of stay in one of the Y-maze branches, where the samples of urine with and without additional compound will be presented. The amount (0,1ml of urine or the mixture) will be presented in perforated glass container. For each female, the longest time in one of two test branches will be treated as the preference determinant. Within each test, the females will be then divided into two categories, according to sample preference.

Experiment technical details

95 Wistar strand inbreed sexually naive female rats and 5 male rats were purchased at the age of 8 weeks, and kept in laboratory for 2-4 weeks for adaptation and estrus synchronization. Males were housed separately, and each 5 females were housed in polypropylene containers, a dimension of 37 • 26 • 17 cm for each, and were provided an adjusted for age amount of food and tap water ad libitum. The housing room was under a reversed 12:12 light:dark photoperiod (lights on at 1900 h) and at the temperature of 23 ± 2 C. For female subjects, their estrous cycle was determined by vaginal smears and 80 healthy female with synchronized 4-days estrus has been chosen for an assay.
A voided urine sample was collected from 5 ten-week old males. Each subject was placed in a clean plastic mouse cage (dimension: 31.8 • 20.2 • 31.5 cm) with a wire grid floor. Upon animal's urination, the urine was immediately absorbed and transferred to a vial by a disposable glass capillary (inter diameter [i.d.] 1.8 mm and 15 cm long) for behavioral and chemical assays. The collected urine samples were individually sealed in vials and kept at –20 C until experiment day.
At the day of expected estrus, the females were divided into 4 groups, 20 in each group. The experiment was conducted at the estrus day, since the 2nd hour after dark.
The experiment will be conducted in four Y-mazes. Each maze size was: 10cm *50cm* 20cm

The prerequisites

The applicant is skilled in laboratory animals handling, participated in the course:
"The breeding and utilization of laboratory animals", and accomplished the courses in zoology, animal physiology, biochemistry. The experiment will be held in the facility of Jagiellonian University Department of Zoology.

8. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

The albino variation of *Rattus norvegicus* is one of the most used animal models in biological research, sharing the characteristics of chemical communication with its wild counterpart- the brown rat, that is considered a pest and disease carrier. Therefore, the attention must be paid to its chemical communication and factors determining sexual attractiveness, to ease further research and provide tools for wild populations' control. This

References

1. Christiansen E., 1976, Pheromones in Small Rodents and Their Potential Use in Pest Control, Proceedings of the 7th Vertebrate Pest Conference (1976). Paper 11
2. Ian Q. Whishaw and Bryan Kolb, 2005, The Behavior of the Laboratory Rat: A Handbook with Tests, Oxford University Press
3. Jian-Xu Hang, Lixing Sun, Jin-Hua Zhang and Zhi-Yong Feng. 2008. Sex- and Gonad-Affecting Scent Compounds and 3 Male Pheromones in Rat. Chem. Senses 33: 611-621
4. Kenji Mori, 2010, Chemical Synthesis of Hormones, Pheromones and Other Bioregulators, Wiley
5. Mark A. Suckow, Steven H. Weisbroth, Craig L. Franklin, 2006, The Laboratory Rat, Elsevier
6. Mossman CA, Drickamer LC, 1996, Odor preferences of female house mice (Mus domesticus) in seminatural enclosures., J Comp Psychol. 1996 Jun;110(2):131-8.
7. Taylor G. T., 1980, Sex pheromones and aggressive behavior in male rats, Animal Learning & Behavior 1980, 8(3), 485-490.
8. Tristram D. Wyatt, 2003, Pheromones and Animal Behaviour, Cambridge University Press

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yy)	Expected cost (zł)
1	Establishment of breeding	10/12	18327
2	Experimental phase and data analysis	11/12	22595
		Total	40 922

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)			
		2012			Total
1	Direct costs, including:				
	1/ Salaries and benefits	6300			6300
	2/ Equipment	20062			20062
	3/ Other direct costs	5060			5060
2	Indirect costs	9500			9500
3	Total costs (1+2)	40 922			40 922

Details of direct cost items
1) Salaries and benefits:
a) salary (two people for two months): 6000 zł
b) salary for 3 assistants (for one day): 300 zł

2) Equipment:
a) rat cages: 145 zł x 20 = 2900 zł
b) litter for rodents (60 l): 10,50 zł x 14 = 147 zł
c) food for rats (1 kg): 7 zł x 40 = 280 zł
d) rats (Wistar Han 8 weeks old): 30zł x 100 = 3000 zł
e) Y-sharped labirynth (Stoelting Any-Maze): 3000 zł x 4 = 12000 zł
f) collecting urine cage: 350 zł
g) DP-930BQ/IRD 540540TVL 6mm camera: 250 zł x 4 = 1000 zł
h) squalene liquid 98% (10 ml): 70 zl
i) 2-heptanone (1 ml): 165 zł
j) 4-ethylophenol (100 mg): 150 zł

4.3.2. Reviews

- Beata Klimek

Review of Project: The composition of urine influence male rat's sexual attractiveness

Proposed research project has on the aim assessing the effect of urine composition on rat male sexual attractiveness. The topic is interesting and concern pheromone communication in animals. However, I do not understand the goal of proposed experiments. Authors give, that there was showed previously, that tested chemicals increased rat male attractiveness to females, even for castrated males. The aim of project is not looking for new compounds nor determining their effective concentrations. In project summary Authors write about proportions between given chemicals, but this potentially new element in their research is not explored in the proposal. I suggest to develop the project description and re-write the whole proposal, with concentration on the aim of the project.

Other remarks.

The title – in my opinion is too indicative; such given title may be adequate for the final manuscript (despite the paper publication is not given as an expected result of this project?).

There was few typing mistakes in text needed correction.

There was no references in the text.

Project schedule has to be composed since 2013y. I suggest longer project duration than one year. A lot of unexpected factors can cause time delay and problems with project final statement. Authors should plan at least few months on data analysis, manuscript preparation and publication.

Budget of project seems to be adequate. Are labyrinth and camera planned to buy as laboratory equipment? If yes, indirect costs of project should be calculated with exclusions of this amount.

Summing up, Authors have to spend more time on their proposal.

- Paulina Kramarz

The proposed study aims to explain not fully understood problem of an influence of chemical communication between sexes on female choice of the most attractive mate. For the scientific value of the study and the clarity of the proposal, the Authors should check spelling (also in the title) as well as the use of proper tense – for example in part 3, paragraph 2 mostly future time should be used. Then, maybe they will find my below remarks improving the proposal.

1. Study species

Please explain why you have chosen Wistar inbreed strain and the chosen age of males,

2. Experimental design

For two-year project four treatments study is rather modest. As you suggest in the Part 1, the most important seems to be proportion of each pheromone, so I would extend the study to more full factorial to get more reliable data. For example, each pheromone can be check in the following way: 25%, 50%, 75% and 100% of studied mixture, plus control and plus replicates (which number is not clearly mentioned in the text). I am aware that it would add some labour but then the results will be valuable and also publishable.

It should be also explained how are you going to prepare the pheromones mixture and how will you remove the given pheromone from the male urine. The last should be checked for the pheromone concentration that means that the budget is lacking of chemical (pheromone) analysis.

One point more – with more complicated design more complicated maze should be consider. And please consider cooperation with Prof. Koteja team as they are equipped with camera and modern software (Noldus) to analyze a behavioral data.

3. Data analysis

There is no single sentence on the subject (only in the Project Summary). But taking into account improving of experimental design proposed by me maybe General or Generalized (depending on the obtained data properties) Linear Model should be applied.

4. Budget

To be honest I like superficial plan of spending money, because you propose not report the study – but please remember that proposal will be checked also by clerks. So you should

specify when you are going to purchase equipment, to analyze data, to prepare the manuscripts.

As I have mentioned above, please remember about including a cost of pheromone measurements. And assuming more labour maybe you should also extend the salary items.

5. Sum up

Perfect proposal for preliminary study but this kind is not funded by any agencies.

- Justyna Gutowska

Review of the grant proposal The composition of urine influence male rat's sexual attractiveness

The hypothesis of the project is not clear. It is stated in the project summary that "some of urine compounds may increase the attractiveness of male rat's urine". At the same time, in the main text it is written that state of knowledge is that "adding any of the 3 compounds separately to castrated male urine (CMU) increased the attractiveness of CMU to females" and that "adding the 3 together to CMU significantly increased the attractiveness" as well.

The hypothesis stated in the summary that "it may occur, that the crucial determinant may be the proportion, not the concentration itself" finds no answer in the experiment design description. It is also written in the summary what statistical analysis is planned, but in the main text we cannot find any specification of this.

The text is slipshod and needs to be revised in terms of linguistic correctness. There is lack of citations in the text. It is not clear how the time in one of two test branches will be measured – with recording the rat's in a maze with camera or live with a stopwatch in a hand.

There are some mistakes in the proposed budget. Materials were classified as equipment (e.g. fish, hygienic materials). At the same time, indirect costs were estimated also from the equipment value.

The issue raised in the project seems interesting, but should be more specified in the details. The grant proposal definitely needs to be corrected in terms of clear problem stating, linguistic correctness, and general arrangement and coherence of the content.

- Michał Kuciel

Revision of the project entitled: 'The composition of urine influence male rat's sexual attractiveness'

The proposed for review project involves the changes to the male's rat urine attractiveness for the females depending on the added pheromonal substances.

The hypothesis is placed correctly but predictions are not very clearly defined, in fact it is mentioned the only in summary.

Abstract is too detailed. Keywords are properly matched. Lack of references both in the description of the problem and in the problem's actual knowledge. In the description of the experiment authors plan to use a particular strain of rats (95 Wistar), but the lack of explanation why this strain. Will purchased males be held in the same room as the females? Is the room in which the females will be kept protected from ingress of the volatile fraction of male urine? What amount of reinforcement will be added to the urine?

The information contained in point. 4 address the point 2.

Lack of expected results of the problem.

Numbers of grammatical errors and occasional typos.

Point 2. of schedule is too general.

Proposed budget: Point 2: b), c), d), h), i), j) should be included in 'other direct costs'.

- Diana Maciąga

Project assessment
The composition of urine influence male rat's sexual attractiveness

Title:

The title is confusing. Is it The composition of urine influences male rat's sexual attractiveness, or should it be The composition of urine's influence on male rat's sexual attractiveness?

The minor grammatical error does not change the meaning fundamentally, but it hinders understanding of the title at first glance. Furthermore, the second option is appropriate for a grant application, while the first one would be more suitable for a paper as it reveals the outcome of an experiment we don't know yet.

Summary:

The description of the experiment is confusing. For a person unfamiliar with the bioassay protocol it is difficult to instantly imagine the distribution of samples between tested groups. I recommend describing control as consisting of two void urine samples, and then moving on to the description of 3 other test combinations.

The term 'void urine' raises my doubts. If this is a common name for such a substance I suggest using this notion from the beginning instead of the 'urine without intervention'. If it is not, perhaps there would be a better world for it as 'void' suggests extracting something from

urine and living the urine that now lacks in this extracted substance. The meaning behind 'level of attractiveness' should be also explained as here it appears only as a term.

Furthermore, it is not clear from the summary whether the role of proportion will be tested or if it serves only as an explanation of possible alternative result.

Additionally, the world 'deviation' should be replaced by 'variation'.

The issues (introduction, hypothesis, methods, expected results) could be divided into paragraphs as this would facilitate reading. The sentence starting with 'It is expected that...urine' fits better into the paragraph explaining the aims. It's next part could be preceded by description of desired results of the experiment.

What problem is proposed:

The authors do not clearly answer the question why this problem should be investigated. However the most disturbing is that the problem of the proportion of the pheromones has no continuation in further parts of the project, especially the experiment design, only 'the state of knowledge' part refers to it indirectly. From the type of data that are to be obtained through the experiment one might imply that a completely different question should be indicated to answer.

Present state of knowledge:

It is difficult to understand this paragraph and information relevance, supposedly due to linguistic errors. The second sentence brakes off, the third is incomprehensible. I suppose the world 'recently' is misused. The same situation occurs in 5th: Here 'it' refers to bioassay, while it shouldn't. In 6th 'as well' seems unnecessary.

If in both experiments adding CMU to urine increases attractiveness, why is the contrasting word 'however' used? Why was the higher than physiological level of concentration used in 1st and not in second study? Did both combinations failed to restore female's preference or just the second one?

The experiment:

I suggest starting with specifying what hypothesis is to be tested. The expected results do not correspond with the question proposed in previous parts of the application.

As it has already been pointed out in case of summary, it would be of great benefit to describe the four test less chaotically. Attaching a table showing what different versions of urine will be used might be considered by the authors. That it is not said what proportion of urine to the additive is used means that it isn't important for the study?

The experiment involves using 5 samples of urine from 5 different males. The question is why 5? In given case how is the possible effect of a male as a factor affecting female choice controlled? Is it not possible that males themselves differ in urine composition and if not – why don't they? Wouldn't it be advisable to use urine from just one male to avoid additional male-related effects?

The information on number of mazes should be also provided. What is the estimated total time of the experiment?

Finally, the description of the maze experiment should not be mixed with the description of the rat breeding details. It would seem reasonable to clearly separate the issues by placing them in different paragraphs.

Although the authors mention in the summary that certain statistical methods will be used, this aspect of data processing is not further developed. Its crucial importance for the project outcome requires that the statistical analysis be described in details.

Last but not least, the researchers haven't conducted the experiment yet. All the 'was' and 'weres' have to be replaced with will! The reason for doing so and possible grave consequences of forgetting it are self explanatory.

Expected results:

The paragraph breaks off rapidly. It does not provide the reader with any information on whether and how the results will be disseminated, which in turn raises doubts about authors' attitude towards the significance of the project. The fact that the possible benefits regarding brown rats control derived from this research has not been mentioned previously is odd as well.

It would be also advisable to use the Latin name Rattus domesticus at the very beginning of the grant proposal, yet it should be accompanied with suitable year and name of a scientist. The scientific name of the model animal should by no means appear in the last part of the text for the first time.

References:

That there is NO single paper actually cited in the grant proposal is unacceptable. In light of this the sole presence of references is unfounded as they do not refer back to anything.

The bibliographical notes need proofreading and standardisation.

Costs:

The dates of completion of the project seem highly unreasonable in view of the length of the grant application proceedings. Furthermore it is doubtful if one month will be sufficient to complete the subsequent parts of the project as both rat breeding and analysing visual data is time consuming.

The reagents, rats, rat food and 'collecting urine' should not be defined as equipment.

Language used:

The language used in most part of the application can be defined as comprehensible, however the text needs thorough proofreading in terms of grammatical and spelling errors. Emphasis should be put especially on the third person adjectives (the 's' ending), the use or lack of prepositions and punctuation. Certain sentences require correction in terms of logic and meaning. This problems are most acutely felt in the paragraph on the present state of knowledge

It is recommended to use the term 'individual' rather then 'specimen' as the latter better fits objects then animals.

Conclusion:

Given that the rat is one of the most heavily researched creature in the world, it is hard to believe that there is room for yet another experiment regarding a trait as popular as its pheromones. However the authors statement that not every problem connected with chemical communication has been investigated sounds convincing. Taking it into consideration, I find the given reasons sufficient to support conducting this research. Nevertheless certain aspects of the experiment should be deeply rethought with the view to redesign the protocol. Moreover, the grant application itself has many flaws and requires and in depth correction. Its shortcomings in terms of language and layout (missing ends of paragraphs, lack of references, language errors) seriously belittle the supposed scientific impact of the experiment and gravely undermine author's credibility.

- Joanna Sudyka

Review of the grant proposal: The composition of urine influence male rat’s sexual attractiveness

The project regarding pheromone role in rats had left me confused. It seems like the authors had something else in mind but they had to introduce rapid changes and they forgot to do it in the whole project. It definitely lacks coherence.

The experiment might have been interesting even if not innovative but the study design appears to be unconsidered. Authors did not state why the problem they undertook is important, they state that they study proportions of pheromone compounds in abstract and

paragraph 1, but later on there is nothing like this even mentioned. Hypothesis are not clearly outlined. For what is mentioned in paragraph 2 - state of knowledge I got an impression that the proposed study issues are already known so there is no need to repeat them.

The statistical issues are mentioned only in abstract (which is not necessary) but methodology is silent on that matter. The t-test seems to be completely inappropriate as there are many more factors involved in experimental design. The costs are incorrectly stated; rats, liter for rodents or reagents should go under other costs and not equipment. Graphically the authors should care for details more (such as removal of spare columns in tables, keywords after summary not after paragraph 1, etc.). It seems that the authors do not want to share their findings with audience - they planned no papers or participation in conferences. There are no appropriate citations in the text, even in the state of knowledge paragraph! There are many unfinished sentences and thoughts (like last one in paragraph 4). Some mistakes in English spelling and grammar (ex. line 14) need more attention. References are not very neat, for instance year of publication is repeated twice in some of them; some have abbreviations of initials and some full first names, dots or comas, etc.

Summing up: The project has much more weak points than strong ones, it should be rethought, starting from reason of undertaking it, through methods, statistics and many formal mistakes. I do not have a clue who was more lost reading it: me or the authors.

4.3.3. Final version of the project

Title changed :
The influence of male rat's urine composition on sexual attractiveness

Project summary
Pheromone play important role in mammal communication. A little has been known about sexual pheromones in rat, despite it is one of the most important model animals in biology. This study aims to determine, if olfactory active compounds of male rat's urine are responsible for its sexual attractiveness. The bioassay with 80 sex-naive female Wistar inbreed rats in estrus phase will be conducted under controlled conditions in Y-maze. The females will be divided into 4 groups and exposed simultaneously to two samples of urine: one without any intervention, another one without (test 1- control) or with additional compound: squalene (test 2), 2-heptanone (test 3), 4-ethyl phenol (test 4). The basic urine sample will comprise of void urine samples of 5 male rats. The preference for one of two samples will be measured as a time the female subject spent in sample proximity. The deviation in urine sample preferences will be tested with t-test, and different attractiveness levels will be tested with Friedman test. It is expected, that some of the chemosignal urine compound may increase the attractiveness of male rat's urine, however it may occur, that the crucial determinant may be the proportion, not the concentration itself, and therefore increasing the level of one compound may have adverse effect on its olfactory attractiveness.

PROJECT DESCRIPTION, METHODOLOGY, AND EXPECTED RESULTS

9. What problem is being proposed and why?

Many mammals use volatile molecules, such as the pheromones, to affect the behavior of other specimens they share their living space with. Pheromones carry the information about species, sex, health status, social position and current stress condition. Pheromones may consist of single compound, but may be also a complex of different molecules. As volatile molecules, the pheromones are very difficult to investigate, especially in quantitative way. Thus, it is difficult to assess the efficacy of single compounds of conjunct pheromones. Such situation occurs in rats, where at least 3 molecules were recently identified as sex pheromones ([2, 3, 7]).

The key question to answer in this research is whether the different proportions of 3 pheromone compounds in the urine may have an impact on attractiveness of a male rat.

Keywords
rat, pheromone, squalene, 2-heptanone, 4-ethyl phenol, sexual attractiveness

10. *What is the present state of knowledge in the field, and to what extent does this project verify it? How will the project advance discovery and understanding in its field or across fields? Is this a new or a continued problem?*

Despite the rat is one of major model animals for biological research, and has been even domesticated for this purpose ([2, 5]), a little is known about chemosignals in this species. Researchers identified some pheromones in rats, including dodecyl propionate, a compound of rat pups preputial gland, that initiates a maternal reaction ([3]).
The voided urine and preputial gland secretion of the rat can convey a information about species, sex, reproductive condition, and stress status ([1, 6, 7, 8]), however, little is known about which compounds are involved in coding for the information recently.
Three compounds were identified as sex pheromones in male rats: squalene, 2-heptanone, and 4-ethyl phenol ([3, 4]). These were identified by a bioassay, as it could restore an attractiveness of castrated rats' urine to sex-naive females. Adding any of the 3 compounds separately (at a concentration higher than its physiological level in male urine) to castrated male urine (CMU) increased the attractiveness of CMU to females, as well. However, adding the 3 together (at the levels in normal male urine) to CMU significantly increased the attractiveness of CMU to females. Nevertheless, such combination did not fully restore females' preference for urine from intact males, suggesting that some other trace compounds might also play some roles in sex attractiveness ([3]).

11. What is the proposed methodology? How will it solve the problem? What equipment will be used? Does the applicant have the required equipment skills and access?

Experiment design and data processing
To test the hypothesis, the bioassay of urine samples will be carried. There will be 4 assays conducted. For each assay, the group of 20 receptive (in estrus) female rats will be the placed

in one branch of Y-maze and left to freely explore the maze for 2 minutes. In each of other two branches, there will be a sample of male rat urine, and the sample of urine without (test 1-control) or with additional compound: squalene (test 2), 2-heptanone (test 3), 4-ethyl phenol (test 4).

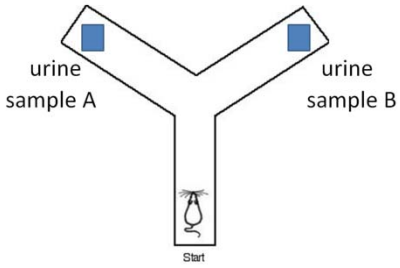

Fig.1. Y-maze

The female rat preference to the particular urine-pheromone mixture was expressed by the time of stay in one of the Y-maze branches, where the samples of urine with and without additional compound will be presented. The amount (0,1ml of urine or the mixture) will be presented in perforated glass container. For each female, the longest time in one of two test branches will be treated as the preference determinant. Within each test, the females will be then divided into two categories, according to sample preference.

Experiment technical details

95 Wistar inbreed sexually naive female rats and 5 male rats will be purchased at the age of 8 weeks, and kept in laboratory for 2-4 weeks for adaptation and estrus synchronization. This strain has been chosen as the most popular and more active than other strains. Males will be housed separately, and each 5 females will be housed in polypropylene containers, a dimension of 37 • 26 • 17 cm for each, and will be provided an adjusted for age amount of food and tap water *ad libitum*. The housing room will be under a reversed 12:12 light: dark photoperiod (lights on at 1900 h) and at the temperature of 23 ± 2 C. For female subjects, their estrous cycle will be determined by vaginal smears and 80 healthy female with synchronized 4-days estrus will be chosen for an assay.
A voided urine sample will be collected from 5 ten-week old males. Each subject will be placed in a clean plastic mouse cage (dimension: 31.8 • 20.2 • 31.5 cm) with a wire grid floor. Upon animal's urination, the urine will be immediately absorbed and transferred to a vial by a disposable glass capillary (inter diameter [i.d.] 1.8 mm and 15 cm long) for behavioral and chemical assays. The collected urine samples will be individually sealed in vials and kept at –20 C until experiment day.
At the day of expected estrus, the females will be divided into 4 groups, 20 in each group. The experiment will be conducted at the estrus day, since the 2nd hour after dark.
The experiment will be conducted in four Y-mazes. Each maze size is: 10cm *50cm* 20cm

The prerequisites

The applicant is skilled in laboratory animals handling, participated in the course: "The breeding and utilization of laboratory animals", and accomplished the courses in zoology, animal physiology and biochemistry. The experiment will be held in the facility of Jagiellonian University Department of Zoology.

12. *What are the expected results of this project ("know-how", patents, methods, equipment), and how will they be disseminated (publications, conference presentations, PhD theses)?*

The albino variation of *Rattus norvegicus* is one of the most used animal models in biological research, sharing the characteristics of chemical communication with its wild counterpart- the brown rat, that is considered a pest and disease carrier. Therefore, the attention must be paid to its chemical communication and factors determining its sexual attractiveness, to ease further research and provide tools for wild populations' control. This experiment will help to determine, if it is the specific composition of volatile signal molecules, what decides about rat urine attractiveness to females, or the attractiveness may be enhanced by increasing the concentration of selected components. With this knowledge, one will be able to control better the reproduction of rat, and enhance the area of research on this species. Besides of related journals, the results shall be presented on the conferences contributing to mammal communication or pest population control, such as Vertebrate Pest Conference, or Chemical Signals in Vertebrates.

References

1. Christiansen E., 1976, Pheromones in Small Rodents and Their Potential Use in Pest Control, Proceedings of the 7th Vertebrate Pest Conference (1976). Paper 11
2. Ian Q. Whishaw and Bryan Kolb, 2005, The Behavior of the Laboratory Rat: A Handbook with Tests, Oxford University Press
3. Jian-Xu Hang, Lixing Sun, Jin-Hua Zhang and Zhi-Yong Feng. 2008. Sex- and Gonad-Affecting Scent Compounds and 3 Male Pheromones in Rat. Chem. Senses 33: 611-621
4. Kenji Mori, 2010, Chemical Synthesis of Hormones, Pheromones and Other Bioregulators, Wiley
5. Mark A. Suckow, Steven H. Weisbroth, Craig L. Franklin, 2006, The Laboratory Rat, Elsevier
6. Mossman CA, Drickamer LC, 1996, Odor preferences of female house mice (Mus domesticus) in seminatural enclosures., J Comp Psychol. 1996 Jun;110(2):131-8.
7. Taylor G. T., 1980, Sex pheromones and aggressive behavior in male rats, Animal Learning & Behavior 1980, 8(3), 485-490.
8. Tristram D. Wyatt, 2003, Pheromones and Animal Behaviour, Cambridge University Press

PROJECT SCHEDULE - ANTICIPATED TASKS

No.	Name and description of task	Expected completion date (mm/yy)	Expected cost (zł)
1	Establishment of breeding	10.2012	18165
2	Experimental phase	06.2013	64290
3	Data processing	09.2013	24000
4	Preparation of papers and conference participation	12.2013	6000
		Total:	112455

PROPOSED BUDGET

No.	Item	Funds for each budget year (zł)		
		2012	2013	Total
1	Direct costs, including:	12975	67350	80325
	1/ Salaries and benefits	6000	49000	55000
	2/ Equipment	5900	13350	19250
	3/ Other direct costs	1075	5000	6075
2	Indirect costs	5190	26940	32130
3	Total costs (1+2)	18165	94290	112455

Details of direct cost items
1) Salaries and benefits:
a) for project managers: 45000 zł
b) for technical assistants: 1000 zł

2) Equipment
a) rat cages: 145 zł x 20 = 2900 zł
b) Y-sharped labirynth (Stoelting Any-Maze): 3000 zł x 4 = 12000 zł
c) collecting urine cage: 350 zł
d) DP-930BQ/IRD 540540TVL 6mm camera: 250 zł x 4 = 1000 zł
e) rats (Wistar Han 8 weeks old): 30zł x 100 = 3000 zł

3) Other direct costs:
a) squalene liquid 98% (10 ml): 70 zl
b) 2-heptanone (1 ml): 165 zł
c) 4-ethylophenol (100 mg): 150 zł
d) litter for rodents (60 l): 10,50 zł x 20 = 210 zł

e) food for rats (1 kg): 7 zł x 60 = 420 zł
f) print the poster: 60 zł
g) abroad conference for project managers: 5000 zł

Author of the photos- Mariusz Cichoń

Workshop participants clockwise from right: Michał Kuciel, Diana Maciąga, Joanna Sudyka, Justyna Gutowska, Damian Kolbe, Ewa Chmielowska.

p


The influence of oxygen concentration
on growth rate in two fish species
differing in the way of oxygen intake


of adult female household cricket
(Acheta domesticus)
on their life strategy.
Diana Maciąga, Justyna Gutowska


The assessment of the effect of
urine composition on rat male
sexual attractiveness

Edited by Ewa Chmielowska

Kraków 2012