



**Review of the doctoral dissertation of Manohara Mahadeva , MTech  
entitled  
*Membrane potential sets the tempo: Bioelectricity controls the rate of  
somitogenesis in chick embryos through mechanics*  
performed at the  
Institute of Animal Reproduction and Food Research  
Polish Academy of Sciences  
in the Team of Reproductive Pathology and Translational Medicine  
in Olsztyn  
under the supervision of Magdalena Kowacz, PhD, DSc  
reviewer: Przemysław M. Płonka, PhD, DSc**

**1. The topic of the dissertation**

*Whether the topic of the dissertation is relevant to degree of the doctor in agricultural sciences in the discipline of animal science and fisheries*

The present reviewer represents widely understood biophysics, an interdisciplinary branch of science, including various aspects of living and dead systems, upon use of physical methodology on various levels of organization. The analyzed doctoral thesis is primarily biophysical and describes physical aspects of organization of organisms during embryogenesis. For the agricultural aspects of the research it is crucial, as well. The development of animal embryos includes the problem of metabolism, increase of mass, and contents of biogenic substances which may in the future turn out human food, and food for livestock. The development of plant organisms is also important to understand the metabolism of herbivores. Physical, biochemical and genetical phenomena described in this thesis including the language of mathematics turn out to be permanent constituents indispensable to model, plan, and design production of food. The described and analyzed experiments are therefore by all means relevant to agricultural sciences in the aspect of zootechnics and fisheries.

The analysis presented in this dissertation is shown in three papers, two of them being already published, and the third one – submitted. They are supplemented by a sort of the dissertation abstract, which sums up the main conclusions and findings, with the accent put on the new observations. The material is divided into three main parts – describing the generation of electric trans-cellular potential, affected by ions (potassium), and pH (resulting from the generation of carbon dioxide) in *Chara*, the model plant organism which in a useful way models the dependence of the potential on the changes in K/CO<sub>2</sub>, and presenting a view of the developing embryo of chicken being also a model for the development of other cultivated animals, and finally, the description of mechanic phenomena resulting from the changeable potential affected by ions and CO<sub>2</sub>. The authors also tries to follow correlations between activation of important developmental genes (genetic pathways) including the Wnt and Pax representatives. If all these phenomena end up in the effect of the development of livestock, it even more manifests itself as the subject for PhD thesis in Agricultural Sciences, in the field of zootechnics.

**2. The candidate's knowledge**

*An assessment, with justification, of whether the dissertation demonstrates the applicant's general theoretical knowledge in the discipline of zootechnics and fisheries*

The Candidate represents a wide and interdisciplinary field of knowledge. Judging from the point of view of a biophysicist, if the dissertation represents biophysics in the agricultural aspects, it must cover many technics and phenomena. The present reviewer can call the name of bioelectricity or electrobiology as the biophysical field of research described in the thesis. It must be abruptly told from bioelectronics, being a rather philosophical branch of knowledge, although maybe in the future found somehow in the described phenomena. Mr Manohara Mahadeva described many important research (and observation) techniques necessary for obtaining and interpret the phenomena controlling chicken embryos, their organization and mechanics. The polarographic measurements of electric fields, their transmembrane potentials and their changes during development deserve a special attention. For the present reviewer they may be associated to the nitoxymetry (measurement of NO), of prof. Tadeusz Malinski from Poznan, which made it able to measure the concentration (activity) of NO in the acting neurons or single cells of smooth muscles. I do not know the effort of Mr. Manohara, but I perfectly realize the procedure of preparing the electrodes for NO-metry and of operating them in the voltametric or amperometric regime. The effort of the PhD candidate in this aspect cannot be overestimated. The Candidate must have understood the interactions between the currents flown through the ionic channels, and affected by changeable electric field (somehow related to the concentration/activity of potassium, carbon dioxide and other factors which should be taken under considerations). These electric fields affect various aspects of neuronal and muscle activity, and here they are the motors for embryogenesis. It was sometimes described by my professors of biophysics who tried in the past describe for the students (and for himself) the action of the so called morphogenetic field, being the result, but at the same time affecting - the position of ion channels in the cellular membranes. This process is fluent, because the membranes are a "fluid mosaic". As a result, the electric field and its potential keeps changing all the time during morphogenesis, and therefore, it was indispensable to find a "stable" model of cells surrounded by cellular wall (*Chara* sp.), to initially provide the way and direction of changes during embryogenesis. Finally, the Candidate must have revealed the detailed knowledge of biomechanics as looking for mechanical changes in the cells and their systems during embryogenesis. These phenomena must have been somehow dependent on the acting geniting "programme" regulated by developmental pathways of signalling. This phenomenon has been tried to be described by Alan Bodnar, who published "A programme of *Drosophila melanogaster*" in which the algorithms regulating the development of a *Drosophila* larvae and the order of phenomena leading to metamorphosis have been described. This is the actual "genetic programme" different of "genetic programming" of artificial intelligence and machine learning. Mr Manohara Mahadeva, by order, has attempted to create a similar programme based on the biophysical phenomena (changes in the membrane potentials) during the development of a chicken embryo. It is a pity that the Bodnar's paper has not been cited in this thesis. Another example of the knowledge and importance of these observations concern the stiffness of melanocytes and melanoma cells, which were described as an important phenomenon for metastasing of melanoma. This tumor contains melanin, which strongly affects the thickness and plasticity of metastasizing cells. It is a pity that the membrane potential of metastasizing melanoma cells have not been measured (yet), but it is a strong temptation to try it in he future.

I must stress out the universal biological and physical knowledge of Mr Manohara Mahadeva, documented this way in the PhD thesis conveyed to Kraków to the present reviewer to be commented and evaluated.

### **3. Independence of the candidate**

*An assessment, together with a justification, of whether the dissertation demonstrates the applicant's ability to carry out scientific work independently*

Mr Manohara Mahadeva has prepared the dissertation in the form of three papers, two of which have already been published. All three papers have been in details described when submitted, and all of them have been reported in the acknowledgements. In all the papers the Candidate prepared and is responsible for – the practical aspects of the experiments. Other authors, including the Promotor, participated in designing the papers and in analyzing the results. The leading role and independence of Mr Mahadeva have also been documented in the statements submitted and included in the PhD Thesis. In all of them Manohara Mahadeva played a crucial role, and all of them (including the third paper which has not been published yet, but is submitted) have been supported by the signs of the co-authors. Nowadays, the scientific work, the experiments in a scientific work are performed always in groups, so it is not unexpected that in the presented papers more than one author participated in the preparation of the results. However, the number of authors listed in the thesis is relatively low (3 authors including the promotor). In the practice of the present reviewer, there are papers submitted for evaluation in the PhD theses, containing up to 6 co-authors, so in the case of the present thesis establishing the main role of the evaluated author, Mr Manohara Mahadeva, is quite easy. It is also evident that the full text of 3 papers preceded by the introduction and ended up with the conclusions and designing the future path of the study have been prepared by the PhD Candidate, which additionally documents his independent authorship and participation.

### **4. Originality of the dissertation**

*An assessment, with justification, of whether the dissertation provides an original solution to a scientific problem / an original solution to the application of the results of one's own research in the economic or social sphere*

Starting from the last point of this aspect of the review, the Candidate prepared the thesis, and carried out the experimental work in the Institute of Animal Reproduction and Food Research representing the branch of agricultural science of PAN, which, by definition and by all the projects represented by the Institute - are of economical/practical value and importance. The described phenomena directly touch the production of food, by studying the mechanisms of embryogenesis of chicken. Their economic and social sphere of research is undoubted. The originality and uniqueness of the thesis may be documented by citations of the two papers which have so far been published, According to Web of Science, they so far gathered 6 citations since 2024, 4 for the paper published in 2024 and 2 – in 2025 (for less than 1 year). To compare, the paper published by the PhD student (already a PhD) and documented his research, accepted and published in 2024, has not been cited yet, though seems to contain important data on NO production in tumors. The papers published by the present Candidate have attracted the interest of the readers immediately and immediately started gathering the citations. In the opinion of the present reviewer, the reason for this interest, besides the practical value of the results, is their typically “biophysical” character – concerning numerous, interdisciplinary aspects of

embryogenesis, manifested by the electrobiological, biochemical, mechanical, and genetical phenomena. It at the same time presents the intricacy, and interdependence of many factors affecting the activity of the organism. It at the same time illustrates in a colourful way the idea of life as a phenomenon. These observations may be, therefore, explored by the representatives of many disciplines and many pathways of thinking, starting from philosophers and evolutionists, through biologists, physicists, biochemists and even mathematicians and IT-specialists who may elaborate the calculable models of these described phenomena, ending up with physicians, food technologists, and veterinarians. In the opinion of the present reviewer, the evaluated Thesis is not only original, but also versatile and universal. It represents the way which has been taken by the creators of biophysicists (e.g. Leo Blumenfeld). Biophysics is a unique discipline which is bound to define the phenomenon of life - to tell the living systems from the dead ones and to describe the process of transformation of one to the other ones. This task is very important in the development of the production of food, and to reach for typically physical procedures to gain this goal is spectacular and original. The evaluated Thesis is a perfect example of this fact.

#### **5. Questions and/or criticisms to which the Reviewer expects the candidate to respond during the defence**

The present reviewer usually treats the preparation of a doctoral thesis evaluation and participation in the defense as an opportunity to learn more about the subject, which to some extent checks the width and interdisciplinarity of the doctoral student's knowledge and skills. Therefore, I would like to raise issues related to, but absent from, the evaluated work. Perhaps this will create an outline or perspective for further research in this area., and to the discussion over the defense.

5.1 I wonder whether the Candidate considered the idea of the inner time, or the subjective time, when investigating the development of embryonic tissue over time. A similar idea prompted prof. Alexis Carrel (Nobel in 1912) to coin the phrase "the inner time" when observing the beating chicken heart muscle in ex vivo cultures, while the chicken would be dead for years. He suggested that the time goes in a different speed for various tissues or cells of an organism. This prompted the theoretical biology to use the differential calculus with delayed argument, very useful e.g. to model the growth of tumor in vivo (in the presence of immunological cells). This idea is very useful to model embryogenesis of chicken embryos on the base of physical parameters as the potential of electric field, and changing the parameters determining the mechanics of these cells in the presence of developing organisms, affecting the embryonic cells via activation of some growth factors (like Wnt/b-Catenin, etc.)

5.2. The observation of somitogenesis is inevitably associated with the phenomenon of involution of muscle (sarcolysis) characteristic for example for metamorphosis of toads who get rid of tail. An important part in this process is taken by melanophores. Sarcolysis is a melanin-involving process. The direction of development is, however, maintained, as in the early phases of embryogenesis. May the Candidate comment on this phenomenon in the context of his experiments? Similar experiments concerning amphibians and their metamorphosis were investigated in Kraków a long time ago, however, in the context of magnetobiology (anyway affecting the electric field and vice versa). The researchers used the new for that time method of measuring spin interactions – the electron paramagnetic resonance. Was modification of the morphogenetic field analysed using EPR?

5.3. As to the morphogenetic field, in the Warsaw institute of PAN a similar investigation aimed at the attempt to invert the polarization of the electric field generated by an organism (and the neural system), thus inducing the so called “electronarcosis” without using any pharmacologic specimens. I wonder if such experiments may be executed in the described protocols – not measuring the geometry of electric field and electric potential by metabolic parameters, but trying to affect the development of embryos by using the external electric field and the accompanying potential.

5.4. Again, in Kraków it was attempted to use the so called “free electrophoresis” to notice the difference in the surface potential between normal and tumor cells in the suspension. I wonder if this free electrophoresis could be useful to distinguish between the developing embryonic cells taken from various places of the embryo, from various stages of their somitogenesis, and/or affected by various factors in solution (the activity of ions, partial pressure of carbon dioxide etc. may affect the process of wandering through the electric field in the free electrophoresis).

5.5. The Candidate have measured and documented the development and changes in the stiffness of somites during embryogenesis of chicken in the subsequent stages of the development. This process has been observed also in the dependence on the concentration or activity of potassium and carbon dioxide. As I have mentioned the electrode of prof. Malinski to measure concentration of NO in cells, it could be very interesting to monitor the changes in NO partial pressure.concentration during somitogenesis of the embryos, or to check the somitogenesis in changeable concentration of NO. Nitric oxide is an important regulator of many [hysiological processes, such experiment might turned out difficult, but nevertheless worth efforts.

## **6. Other observations on the content or form of the dissertation (optional)**

In the opinion of the present reviewer here there is a place to pinpoint some improprieties observed during the lecture of the PhD thesis.

- This paper is well written, but there are some letter mistakes observed, one has to double check the text before sending it further.
- I have got used to prepare the aims and the conclusions of a text as the itemized list of several most important ones, and so as every conclusion were the reference of one aim listed in the beginning of the thesis. I could not find such relation in this document.
- The conclusions seem in this document repeat the discussion (in a shorter form) which is a tautology.
- Some results of measurements are listed in the text “in prose”, accompanied by the SD. When reading the full text, such construction makes the reading difficult, and the main plot difficult to follow.
- General discussion, general conclusions and plans for the future seem to be overlapped and repeated one after another to some degree. It might have been shorter.
- The list of references is not numbered, that is a pity, one cannot say at a sight how many papers have been cited in this text. The author gained about 250 citations, but it is not a precise number.
- The statistics of these experiments has been executed, but in my personal opinion the statistics is an additional tool for research. For example, the statistical difference between variations of particular groups of parameters were an important parameter (like in the ecology) prompting the heterogeneity of the population of parameters, moreover, another (nonparametric) test for the differences of measured parameters might then be used.

On the other hand, Mr Manohara Mahadeva devoted a couple of lines to describe his position in the science. It is striking and remarkable, that his intention to make science, to investigate the phenomena of the reality, started in the early childhood, and has accompanied his life, which must have resulted in such an original attitude to the phenomenon of life.

## **7. Final evaluation**

I, the undersigned, state that the reviewed doctoral dissertation of **M.Tech. Manohara Mahadeva** entitled **Membrane potential sets the tempo: Bioelectricity controls the rate of somitogenesis in chick embryos through mechanics** meets the conditions set out in Article 187 of the Act of July 20, 2018. Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended) and I request the Scientific Council of the **Institute of Animal Reproduction and Food Research Polish Academy of Sciences** in Olsztyn to admit Manohara Mahadeva, M.Tech, to further stages of the procedure for awarding him a doctoral degree in the field of Agricultural Sciences in the discipline of animal science and fisheries.

I, hereby, request that the thesis is accepted with distinctions

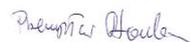
In the opinion of the present reviewer, PhD student Manohara Mahadeva deserves to be awarded the doctoral degree with distinctions.

He published his doctoral dissertation entitled **Membrane potential sets the tempo: Bioelectricity controls the rate of somitogenesis in chick embryos through mechanics** in the form of 3 experimental papers, 2 of which were published and 1 has been submitted.

1. These papers have been submitted or published very recently (2024 and 2025) and gathered already 6 citations in journals enjoying IF over 2. The published ones represent Biophysical Journal and Developmental Biology, both of Elsevier.
2. The experimental field of the Candidate is very interdisciplinary, which betrays the fact of versatility of the Candidate in knowledge and skills.
3. The subject touches the very important field of interest, concerning biology, physics and agriculture, therefore implying practical, applicative aspects of the dissertation
4. From the point of view of the present reviewer, a biophysicist, the dissertation touches the crucial point of biophysics, i.e. concerning the definition of life, as a phenomenon, and as a process. This may affect the development of various disciplines of science, at the same time - the hard sciences, social sciences and to some degree humanities.

Kraków, January 16, 2026

.....  
date



.....  
Reviewer's signature

Przemysław M. Płonka, PhD, DSc  
Department of Biophysics and Cancer Biology  
Faculty of Biochemistry, Biophysics and Biotechnology  
Jagiellonian University in Kraków