Faculty of Science
Zoology Department

Molecular and Histocomparative Studies on Seasonal Variations among Frogs

By

Shaimaa Shaban Mahfouz Abd El-Aty

B.Sc., in Zoology & Chemistry, 2017

Faculty of Science, Fayoum

University.

A Thesis
Submitted for Fulfillment of
The Requirements for M. SC. Degree
In Zoology

(Cytology & Histology)

From
Zoology Department,
Faculty of Science.

FAYOUM UNIVERSITY

2023

Faculty of Science
Zoology Department

Molecular and Histocomparative Studies on Seasonal Variations among Frogs

By

Shaimaa Shaban Mahfouz Abd El-Aty

B.Sc. in Zoology & Chemistry 2017

Faculty of Science
Fayoum University

Supervisors:

1. **Prof. Dr. Abdelkarim Mohammed Abdellatif**

Professor of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

2. **Dr. Ayman Mostafa Abdelaziz**

Assistant professor of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

3. **Dr. Heba Mohammed Rabeae**

Lecturer of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

Vice Dean of Postgraduate Studies and Research
Fayoum University

Prof Dr. Somia Elsaid Goda

Approval Sheet

Molecular and Histocomparative Studies on Seasonal Variations among Frogs

Name of candidate:

Shaimaa Shaban Mahfouz Abd El-Aty

(B.Sc. in Zoology / Chemistry, 2017)Faculty of Science
Fayoum University

This thesis for M.Sc. degree has been

Approved by:

Supervision Committee

1. **Prof. Dr. Abdelkarim Mohammed Abdellatif**

   Professor of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

2. **Dr. Ayman Mostafa Abdelaziz**

   Assistant professor of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

3. **Dr. Heba Mohammed Rabeae**

   Lecturer of Cytology & Histology, Zoology Department, Faculty of Science, Fayoum University

SUMMARY AND CONCLUSION

Global warming and climatic changes have a major effect on animal species, particularly amphibians. Many of these effects have adverse consequences for amphibians. Climate changes have an impact on amphibian activity during the spring and winter. Climate change has become a major threat to species around the world over the past few decades. Global extinction rates are currently thought to be up to 1000 times greater than the background rate found in the fossil record. Amphibians are the group among them with the highest percentage of threatened species. Amphibians are significantly impacted by global warming, which causes them to emerge from hibernation earlier.

Cold temperatures and the scarcity of food during the winter lead the grass frog *Rana mascareniensis* and the marsh frog *Rana ridibunda* to hibernate in order to survive. Hibernation is a naturally occurring phenomenon in many animals that enables them to survive harsh environmental conditions. During hibernation heart, metabolic and respiration rates decrease.

The present work aimed to investigate the cytoarchitecture of brain sub-regions affected by the natural thermal cycle's fluctuations during the active period in summer and hibernation in the winter in two species of frogs, the grass frog *Rana mascareniensis* and the marsh frog *Rana ridibunda*.

Both *R. mascareniensis* and *R. ridibunda* frogs were randomly captured by hand during the period of both the active period in suumer (1^{st} week of September 2019) and during hibernation in the winter (4^{th} week of January 2020) from different localities at Abo Roash and El Mansuriya, El-Giza province, Egypt. All collected frog samples were

transported to the laboratory of research at Zoology Department, Faculty of Science, Fayoum University, Egypt.

Animals were anesthetized with intraperitoneal injection with 80 mg/kg sodium pentobarbital then we dissect frogs and isolate the whole brains by this method the frog was attached to the dissection dish with its dorsal side facing up. The skin behind the head was cut crosswise, and using the scalpel, it was pulled forward to be removed from above the head area. The scalpel's edge was then placed above the dorsal midline of the skull, and it was moved backward and forward several times with minimal pressure till the cranium was opened, allowing the edge to raise the roof cranium bones and cut it to reveal the brain. Then we fixed the whole brains in neutral buffered formalin for histological and immunohistochemical studies. But, the olfactory bulbs were preserved in RNA later and stored in 4°C for mRNA extraction and real time qPCR.

All procedures sharing in this work have been approved and authorized by the Institutional Animal Care and Use Committee for Fayoum University (**FU-IACUC; AEC 2204**).

Anatomical results revealed that brain of the two studied species *R. mascareniensis* and *R. ridibunda* is consist of two olfactory bulbs, two cerebral hemispheres, the diencephalon, two optic lobes, the cerebellum and the rhombencephalon.

Brain sub-regions showed normal histological structure in the two studied species during the active period in summer using toluidine blue stain. In contrast, degenerated, vasogenic neurons with perivascular space, neurons with condensed chromatin and neurons with inverted axons and degenerated axons were observed in dorsal pallium, medial pallium, lateral septum, medial amygdala, central amygdala, dorsal and ventral thalamus, hypothalamus, optic tectum, optic tectum layers,

tegmentum, granular layer of cerebellum, trigeminal motor nucleus, facial motor nucleus and main abducens nucleus in the brain of *R. mascareniensis and R. ridibunda* during hibernation in the winter.

Immunohistochemical technique revealed high levels of Na^+/ K^+-ATPase expression in different brain sub-regions during the active period in summer in the two studied species which reflect the importance of high Na^+/K^+-ATPase for the metabolic regulation processes to maintain the different brain functions activities. In contrast, low levels of Na^+/ K^+-ATPase expression in different brain sub-regions during hibernation in the winter in the two studied species due to the decreased neuronal excitability and the impairments of cognitive functions and for energy conservation.

Also, immunohistochemical technique revealed high levels of pax6 expression in different brain sub-regions during the active period in summer in the two studied species which may reflect its importance in the proliferation and regeneration processes to compensate the degenerated neuronal cells in the different brain sub-regions occurred during hibernated period. In contrast, low levels of pax6 expression in different brain sub-regions during hibernation in the winter in the two studied species indicating low regenerative activity.

Using RT-qPCR we have documented the up-regulation of a number of genes in olfactory bulb tissues of the two studied species during hibernation in the winter including heat shock protein (HSP30, HSP40, HSP47, HSP70, HSP90 and HSP110) suggesting that these chaperones may play an important role in protecting and stabilizing proteins at low temperature, up-regulation of uncoupling protein UCP1suggesting that this protein may play an important role in thermal

adaptation of brain, up-regulation of UCP2 and metallothionein genes suggesting that these proteins may have a protective effect against reactive oxygen species.

This study show that brain adaptations to low temperature play a crucial role in coordinating stress responses and the importance of olfactory bulb role in the sensation of external temperature fluctuations and thermoregulation. Also, neuronal energy production and regeneration activities among the two studied frog species are markedly reduced with decreasing body temperature which appear in our study through the decreased expression of Na^{+}/K^{+}-ATPase as energy regulator and Pax6 as transcriptional factor during hibernation in the winter.

Additionally, the molecular expression of uncoupling proteins and metallothionein expressed their activity during hibernation in winter to defend against any dysfunctions and protect against oxidative stress. These results have been accessed through the high sensation activities of the olfactory bulb that induce thermoregulatory actions to the other brain sub-regions to carry out their mechanistic role. Therefore, the present study gives the chance to evaluate brain activity during low temperatures and its consequences.

In conclusion, the results of the current study reflect the effects of temperature fluctuation on different studied brain parameters indicating the crucial role of the brain during frog adaptation. Also, reflect the importance of olfactory bulb in the sensation of external temperature fluctuations and thermoregulation.

Finally, the author recommends that other Egyptian frog species should be studied with the use of other genes for molecular measurements and immunohistochemical examination of other proteins to determine whether they follow the same mechanisms during external temperature

fluctuation or not. Also, the necessity to support decision-making processes at the local, national, and international levels concerned with managing climate change because climate change undermines efforts to conserve biodiversity and its sustainable use.