

## Plan Overview

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*A Data Management Plan created using DMP Tool*

**Title:** Endocrine and molecular mechanisms involved in the sexual differentiation of medaka (*Oryzias latipes*) under conditions of thermal stress

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### Project abstract:

The fish present mechanisms of determination and sexual differentiation, both as a genetic and environmental factor, such as temperature variations. These variations may influence the endocrine control of various hormones, such as thyroid hormone (HT), which is characterized by playing fundamental and modulatory roles in response to stress. Therefore, our objectives were to test whether the increase in temperature could modulate endocrine mechanisms dependent on glucocorticoids and thyroid hormones, influencing the expression of genes involved in sexual differentiation in Medaka. Thus, our objectives were to characterize the expression pattern of genes involved in the process of male and female sex differentiation in medaka, to test whether the increase in temperature modulates the expression of these genes, as well as, the expression of genes involved in the synthesis and action of hormones thyroid. The expression pattern in control individuals shows a high expression of *amhrII*, *ar* and *gsdf* genes in males and *cyp19a1* and *foxl2* in females. The incubation of medaka under high temperatures (32°C) induced sex reversal from genotypic females (XX) to phenotypic males (27.3%), increasing the expression of *amh*, *amhrII*, *gsdf*, *ar* in males and females and decreasing *cyp19a1a* and *foxl2* in females. In addition, the expression profile of genes involved in the synthesis (*dioI*, *dioII*, *dioIII*) and the action of HTs (*trh $\alpha$* , *trh $\beta$*  and *tshr*) in both sex were also altered. In this sense, it is postulated that these genes have thermo-sensitive responses when subjected to environmental factors such as thermal stress and that any changes in their expression profiles may reflect changes in the homeostasis of the HPT axis. Future analyzes are necessary to measure the plasma levels of HTs under the effect of thermal stress, as well as the administration of exogenous thyroid hormones and / or blocking the production of HTs by inhibitors. Finally, this study will help to elucidate possible causal relationships between temperature and thyroid hormones during sexual differentiation in this model species, expanding our

knowledge about the regulatory genes of the sexual determination / differentiation cascade in vertebrates.

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# **Endocrine and molecular mechanisms involved in the sexual differentiation of medaka (*Oryzias latipes*) under conditions of thermal stress**

## **Data Collection**

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### **What data will you collect or create?**

- Data on sex differentiation
- Expression profile of genes involved in sex differentiation and in synthesis and regulation of thyroid hormones (qPCR)
- Sex Reversal
- Histological data
- All the data was collected in different experiments.

### **How will the data be collected or created?**

Data will be collected at each experiment and tabulated in Excel spreadsheets. Data analysis will be performed using Graphpad software.

## **Documentation and Metadata**

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### **What documentation and metadata will accompany the data?**

Protocols and detailed notes on each experiment will be recorded in the laboratory notebooks

## **Ethics and Legal Compliance**

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### **How will you manage any ethical issues?**

The Project was submitted to the Ethics Committee on the Use of Animals (CEUA) - UNESP number 1168.

### **How will you manage copyright and Intellectual Property Rights (IP/IPR) issues?**

There are no legal or ethical restrictions. However, the data will be kept confidential until publication in scientific journals.

## **Storage and Backup**

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### **How will the data be stored and backed up during the research?**

The data will be stored by principal investigators in the cloud and external hard drives, with daily backups, and with restricted access to the researchers team involved in the project. In addition, data and

metadata will be published through a platform made available by UNESP, available at <https://repositorio.unesp.br/>

### **How will you manage access and security?**

UNESP will be responsible for your safety for a specified period. UNESP will provide the platform: <https://repositorio.unesp.br/>

## **Selection and Preservation**

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### **Which data are of long-term value and should be retained, shared, and/or preserved?**

Protocol's adaptations of sex reversal must be shared and preserved.

### **What is the long-term preservation plan for the dataset?**

Data sets with long-term value will be preserved in the cloud and on external hard drives.

## **Data Sharing**

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### **How will you share the data?**

Data will be shared with the scientific community through publication in high impact international journals.

### **Are any restrictions on data sharing required?**

There are no legal or ethical restrictions. However, the data will be kept confidential until publication in scientific journals.

## **Responsibilities and Resources**

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### **Who will be responsible for data management?**

Principal investigator, researcher team, UNESP

### **What resources will you require to deliver your plan?**

The principal investigator will keep all data updates, as well as backups, and may request technical assistance from UNESP's IT sector.

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