MASTER RESEARCH PROJECT PROPOSAL

Systemic and mucosal immune response of Nile tilapia broodstock to monovalent and bivalent vaccines against bacteria *Streptococcus agalactiae* and *Aeromonas veronii*.

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Nile tilapia Aeromonas veronii / Streptococcus agalactiae infections

(Left) *Red hybrid tilapia juvenile that positive to TiLV and Aeromonas veronii showing* ***skin redness and hemorrhages*** *around the operculum area, body and base of dorsal, caudal and anal fins.*

(Right) *Black Nile tilapia displaying* ***exophthalmia*** *(pop-eye) and corneal opacity after infection with Streptococcus agalactiae.*

Executive summary

- There is currently no vaccine against the 2 pathogens available to farmers in Thailand.
- Several disease outbreaks have been reported in the past, mostly during hot seasons and after the diminution of DO in the water.
- This research project aims to characterize the immune response of the Nile tilapia to inactivated vaccines for the 2 pathogens ***Aeromonas veronii (Av) and Streptococcus agalactiae (Sa)***
  - Efficacy of 3 vaccines (monovalent Sa, monovalent Av and bivalent Sa+Av) will be assessed
  - In juveniles and in chiltralada 4 broodstock
- Results of the efficacy-indicating study will be published.

Building the research project

Bi-weekly progress reporting

Initiation

- **Review** existing literature on vaccination using inactivated vaccines and antibody response and immunology of cichlids.
- **Conceptualization** : ideas; formulation or evolution of overarching research goals and aims.
- **Funding acquisition** : acquisition of the financial support for the project leading to this publication.
- **Project administration** : management and coordination responsibility for the research activity planning and execution.

Planning

- **Breakdown of project work** in phases, define milestones and deliverables
- **Define methodology** to be used during each project phase
- **Prepare materials** list (fish, vaccine sample, raw material for vaccine production)
- **Lab facilities access** if external to AIT (SSRU, Mahidol Centex Shrimp)

Executing

- **Protocols writing** for vaccine development, ELISA/PCRs and challenge tests
- **Husbandry** and pond preparation
- **Monitoring fish immune response after vaccination**
- **Execution** of the challenge tests and conclude on vaccine efficacy

Closing

- **Write master thesis**
- **Publish one article in aquaculture journals**

Work packages and tasks

- NOT STARTED
- ON-GOING
- COMPLETED
- AT RISK

WP1 - Pond, fish and husbandry

- **TASK1.1**: Prepare pond, stock breeders, select feeding regime
- **TASK1.2:** Feed the offspring and raise 400 juveniles
- **TASK 1.3:** Quality control, fish free of diseases
- **TASK1.4:** Prepare 4 medium experimental ponds for Ct, Sa, Av, Sa+Av
- **TASK1.5:** Acclimation 10-14 days
- **TASK1.6:** Prepare at least 9 small ponds for the challenge test

WP2 - Bacterial preparation, vaccine preparation

- **TASK2.1:** Recovery of the two strains, amplification and culture
- **TASK2.2:** Stockage of bacteria for challenge test
- **TASK2.3**: Production of monovalent vaccine Sa
- **TASK2.4:** Production of vaccine Av
- **TASK2.5:** Production of vaccine Sa + Av
- **TASK2.6:** Production of vaccine Control

WP3 - Immunization and fish swabs

- **TASK3.1**: Fish swab before immunization - (baseline IgM and IgT titres)
- **TASK3.2:** Immunization (Sa, Av, Sa + Av, Control)
- **TASK3.3:** Weekly fish swabs to collect biomarkers

WP4 – Lab assays to monitor immune response

- **TASK4.1:** ELISA for each group of samples for IgM and IgT
- **TASK4.2:** PCR of key immune genes
- **TASK 4.3:** Agglutination tests
- **TASK4.4:** Collect and analyse results
- **TASK4.5:** Draw conclusions

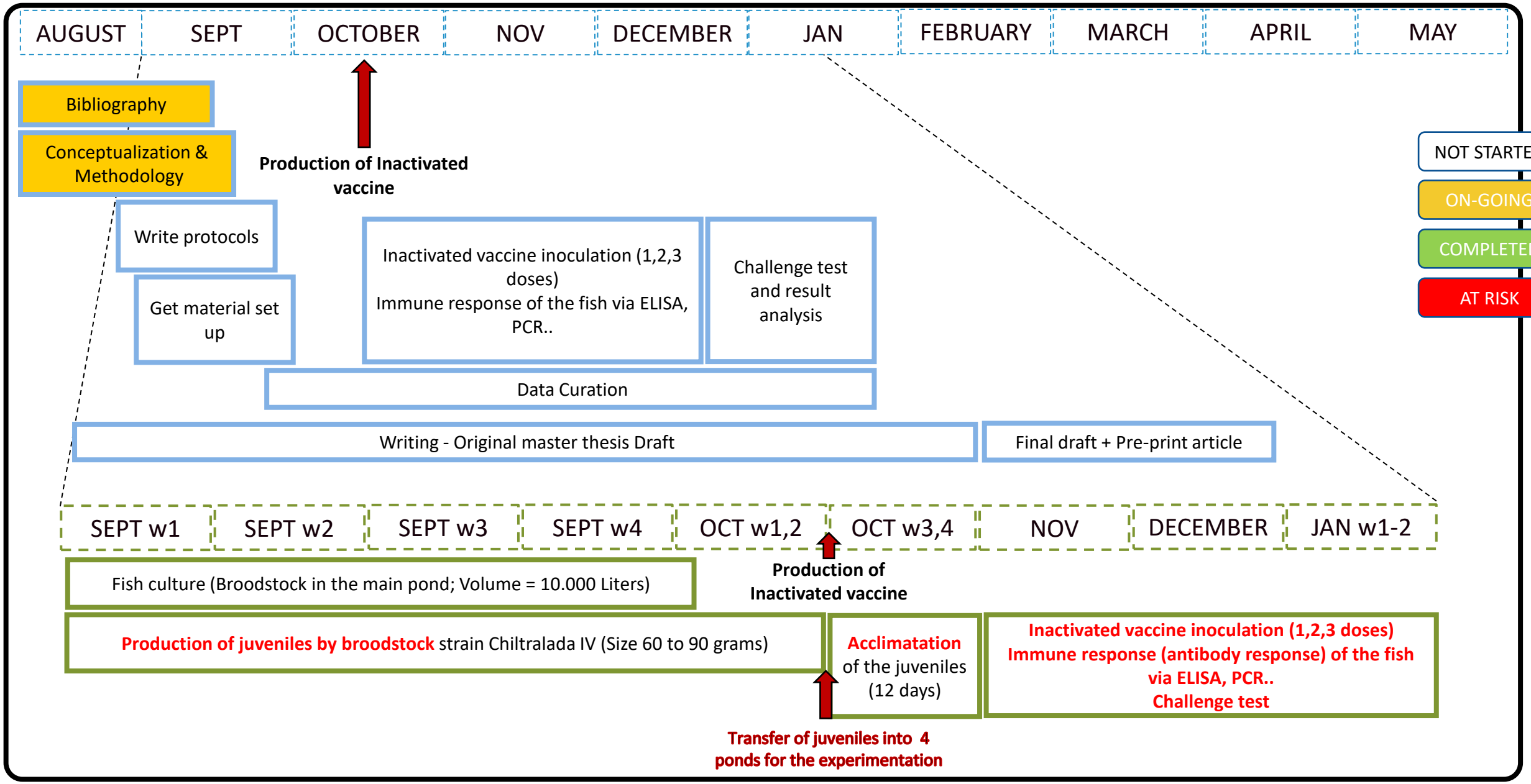
WP5 - Challenge test

- **TASK5.1:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa
- **TASK5.2:** Expose to PBS the group vaccinated with Sa
- **TASK5.3:** Expose to *Aeromonas veronii* the group vaccinated with Av
- **TASK 5.4:** Expose to PBS the group vaccinated with Av
- **TASK5.5:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa + Av
- **TASK5.6:** Expose to *Aeromonas veronii* the group vaccinated with Sa + Av
- **TASK5.7:** Expose to PBS the group vaccinated with Sa + Av
- **TASK 5.8:** Draw conclusions

WP6 - Ethics requirements

- **TASK6.1:** Approve use of fish for the study by Animal Care and Use Committee
- **TASK6.2:** Ensure animal welfare during experimentation

Research project high level schedule



Thank you !