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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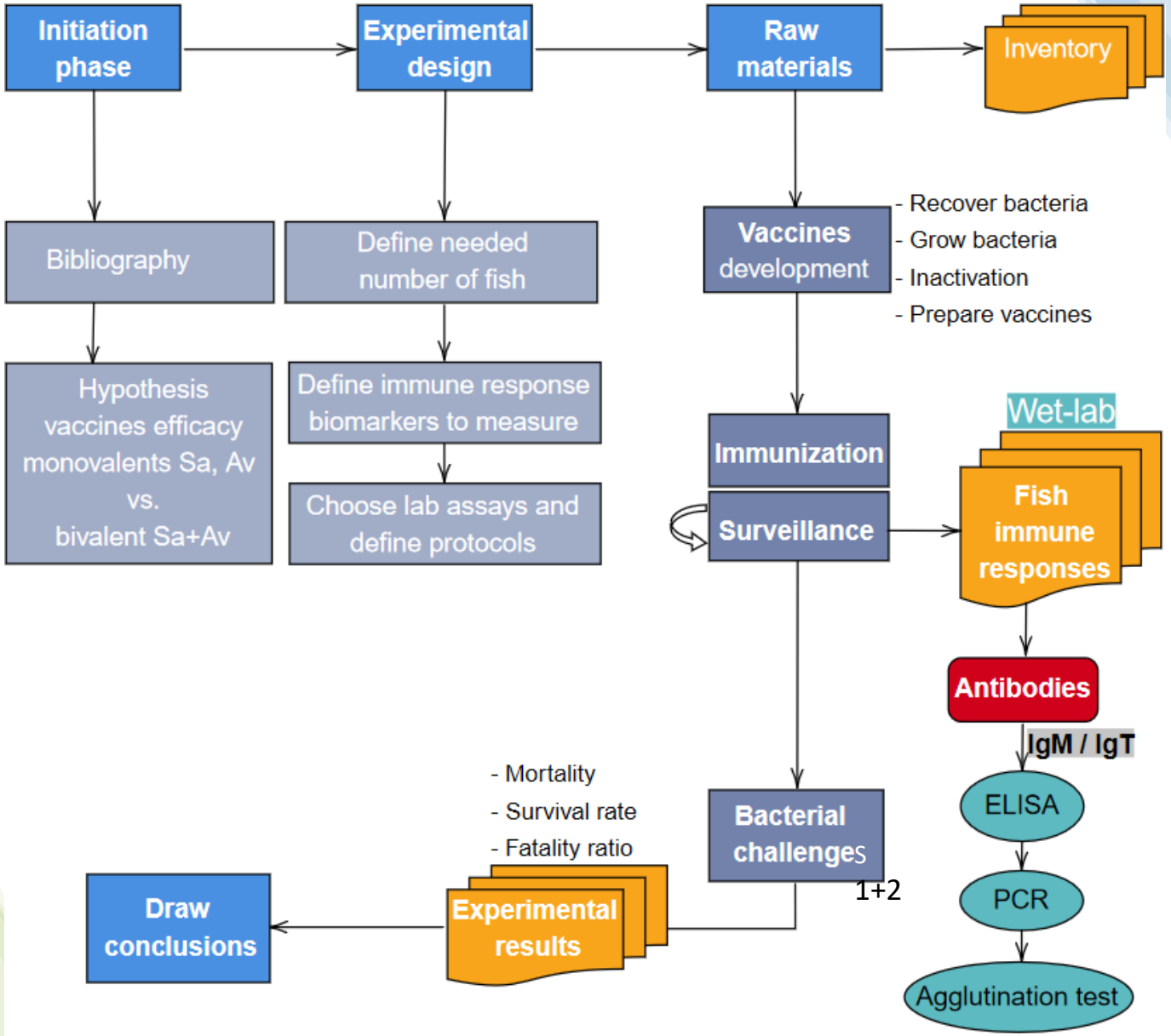
Conceptual framework



Work packages and tasks

WP1 - Pond, fish and husbandry

- **TASK 1.1**: Prepare the 4 aquaria.
- **TASK 1.2:** Assess population distribution parameters
- **TASK 1.3:** Stock 120 juveniles fish of 12-13 grams per aquarium.
- **TASK 1.4:** Record water parameter.
- **TASK 1.5:** Define feeding regime.
- **TASK 1.6:** Acclimate 10 days.
- **TASK 1.7:** Bacterial isolation: fish is free of diseases

WP2 - Bacterial preparation, vaccine preparation

- **TASK 2.1:** Recovery of the two strains
- **TASK 2.2:** Stockage of bacteria for challenge test
- **TASK 2.3**: Production of vaccine Sa
- **TASK 2.4:** Production of vaccine Av
- **TASK 2.5:** Production of vaccine Sa + Av
- **TASK 2.6:** Production of vaccine Control

WP3 - Immunization and fish swabs

- **TASK 3.1**: Fish swab before immunization - (baseline IgM)
- **TASK 3.2:** Immunization (Sa, Av, Sa + Av, Control)
- **TASK 3.3-3.5:** Weekly fish swabs to collect biomarkers
- **TASK 3.6:** Immunization booster (Sa, Av, Sa + Av, Control)
- **TASK 3.7-3.14:** Weekly fish swabs to collect biomarkers

WP4 – Lab assays to monitor immune response

- **TASK 4.1:** ELISA for each group of samples for IgM and IgT
- **TASK 4.2:** PCR of key immune genes
- **TASK 4.3:** Agglutination tests
- **TASK 4.4:** Collect and analyse results
- **TASK 4.5:** Draw conclusions

WP5- Challenge test week 4 post vaccination

- **TASK 5.1:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa
- **TASK 5.2:** Expose to PBS the group vaccinated with Sa
- **TASK 5.3:** Expose to *Aeromonas veronii* the group vaccinated with Av
- **TASK 5.4:** Expose to PBS the group vaccinated with Av
- **TASK 5.5:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa + Av
- **TASK 5.6:** Expose to *Aeromonas veronii* the group vaccinated with Sa + Av
- **TASK 5.7:** Expose to PBS the group vaccinated with Sa + Av
- **TASK 5.8:** Daily monitoring survival rate
- **TASK 5.9:** Draw conclusions

WP5'- Challenge test (end of experiment)

- **TASK 5.1:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa
- **TASK 5.2:** Expose to PBS the group vaccinated with Sa
- **TASK 5.3:** Expose to *Aeromonas veronii* the group vaccinated with Av
- **TASK 5.4:** Expose to PBS the group vaccinated with Av
- **TASK 5.5:** Expose to *Steptoccocus agalactiae* the group vaccinated with Sa + Av
- **TASK 5.6:** Expose to *Aeromonas veronii* the group vaccinated with Sa + Av
- **TASK 5.7:** Expose to PBS the group vaccinated with Sa + Av
- **TASK 5.8:** Daily monitoring survival rate
- **TASK 5.9:** Draw conclusions

WP6 - Ethics requirements

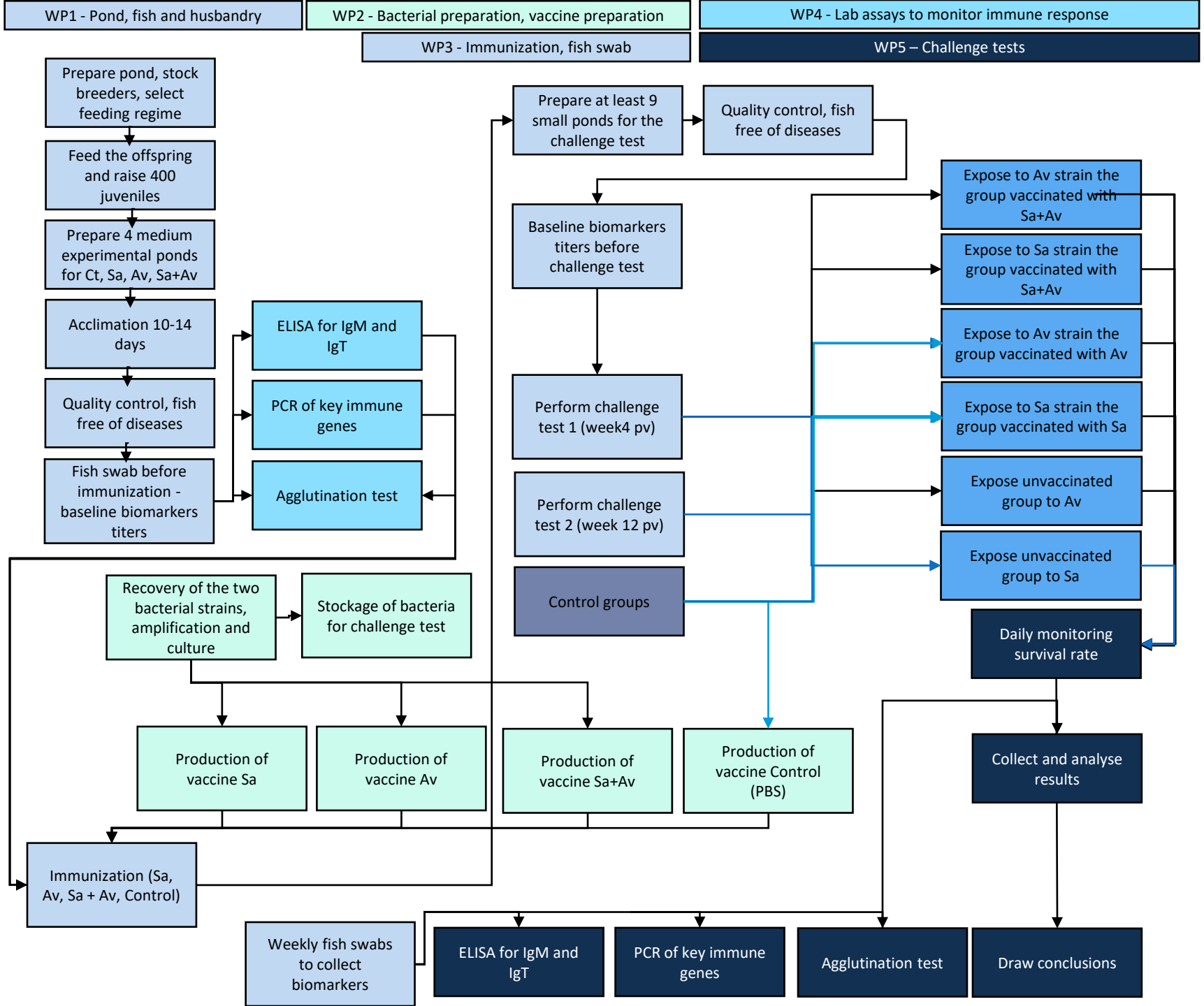
- **TASK 6.1:** Approve use of fish for the study by Animal Care and Use Committee
- **TASK 6.2:** Ensure animal welfare during experimentation

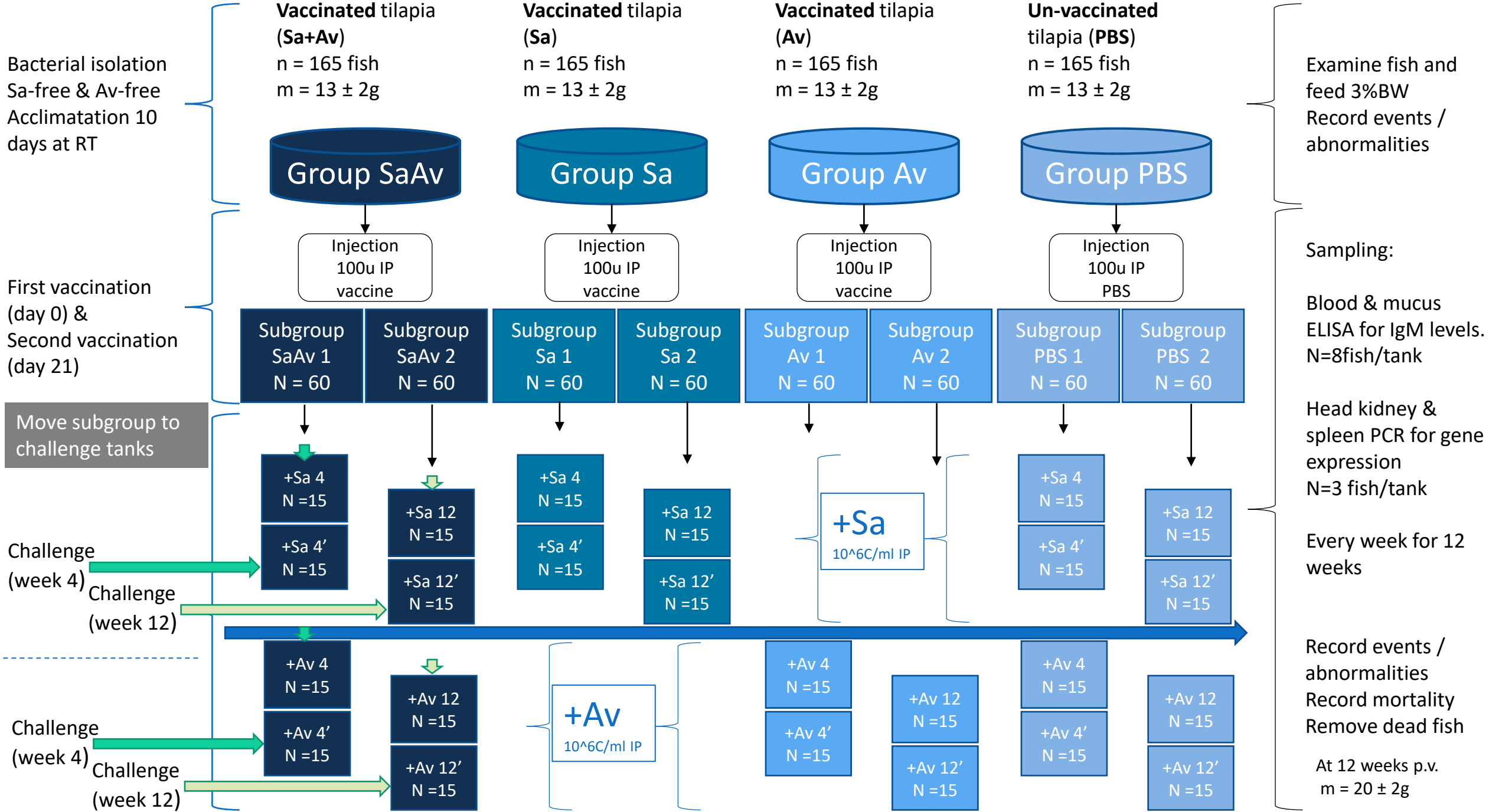
NOT STARTED

ON-GOING

COMPLETED

AT RISK


WP1 - Pond, fish and husbandry
WP2 - Bacterial preparation, vaccine preparation
WP3 - Immunization, fish swab
WP4 - Lab assays to monitor immune response
WP5 – Challenge tests
Prepare pond, stock breeders, select feeding regime
Feed the offspring and raise 400 juveniles
Prepare 4 medium experimental ponds for Ct, Sa, Av, Sa+Av
Acclimation 10-14 days
Quality control, fish free of diseases
Fish swab before immunization - baseline biomarkers titers
ELISA for IgM and IgT
PCR of key immune genes
Agglutination test
Prepare at least 9 small ponds for the challenge test
Quality control, fish free of diseases
Baseline biomarkers titers before challenge test
Perform challenge test 1 (week4 pv)
Perform challenge test 2 (week 12 pv)
Control groups
Expose to Av strain the group vaccinated with Sa+Av
Expose to Sa strain the group vaccinated with Sa+Av
Expose to Av strain the group vaccinated with Av
Expose to Sa strain the group vaccinated with Sa
Expose unvaccinated group to Av
Expose unvaccinated group to Sa
Daily monitoring survival rate
Recovery of the two bacterial strains, amplification and culture
Stockage of bacteria for challenge test
Production of vaccine Sa
Production of vaccine Av
Production of vaccine Sa+Av
Production of vaccine Control (PBS)
Collect and analyse results
Immunization (Sa, Av, Sa + Av, Control)
Weekly fish swabs to collect biomarkers
ELISA for IgM and IgT
PCR of key immune genes
Agglutination test
Draw conclusions


Bacterial isolation
Sa-free & Av-free
Acclimatation 10 days at RT
Vaccinated tilapia (Sa+Av)
n = 165 fish
m = 13 ± 2g
Vaccinated tilapia (Sa)
n = 165 fish
m = 13 ± 2g
Vaccinated tilapia (Av)
n = 165 fish
m = 13 ± 2g
Un-vaccinated tilapia (PBS)
n = 165 fish
m = 13 ± 2g
Examine fish and feed 3%BW
Record events / abnormalities
Group SaAv
Group Sa
Group Av
Group PBS
First vaccination (day 0) &
Second vaccination (day 21)
Injection 100u IP vaccine
Injection 100u IP vaccine
Injection 100u IP vaccine
Injection 100u IP PBS
Subgroup SaAv 1 N = 60
Subgroup SaAv 2 N = 60
Subgroup Sa 1 N = 60
Subgroup Sa 2 N = 60
Subgroup Av 1 N = 60
Subgroup Av 2 N = 60
Subgroup PBS 1 N = 60
Subgroup PBS 2 N = 60
Move subgroup to challenge tanks
+Sa 4 N =15
+Sa 4' N =15
+Sa 12 N =15
+Sa 12' N =15
+Sa 4 N =15
+Sa 4' N =15
+Sa 12 N =15
+Sa 12' N =15
+Sa 10^6C/ml IP
+Sa 4 N =15
+Sa 4' N =15
+Sa 12 N =15
+Sa 12' N =15
Challenge (week 4)
Challenge (week 12)
+Av 4 N =15
+Av 4' N =15
+Av 12 N =15
+Av 12' N =15
+Av 10^6C/ml IP
+Av 4 N =15
+Av 4' N =15
+Av 12 N =15
+Av 12' N =15
+Av 4 N =15
+Av 4' N =15
+Av 12 N =15
+Av 12' N =15
Challenge (week 4)
Challenge (week 12)
Sampling:
Blood & mucus ELISA for IgM levels. N=8fish/tank
Head kidney & spleen PCR for gene expression N=3 fish/tank
Every week for 12 weeks
Record events / abnormalities
Record mortality
Remove dead fish
At 12 weeks p.v.
m = 20 ± 2g

Thank you !