# MASTER RESEARCH PROJECT PROPOSAL

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

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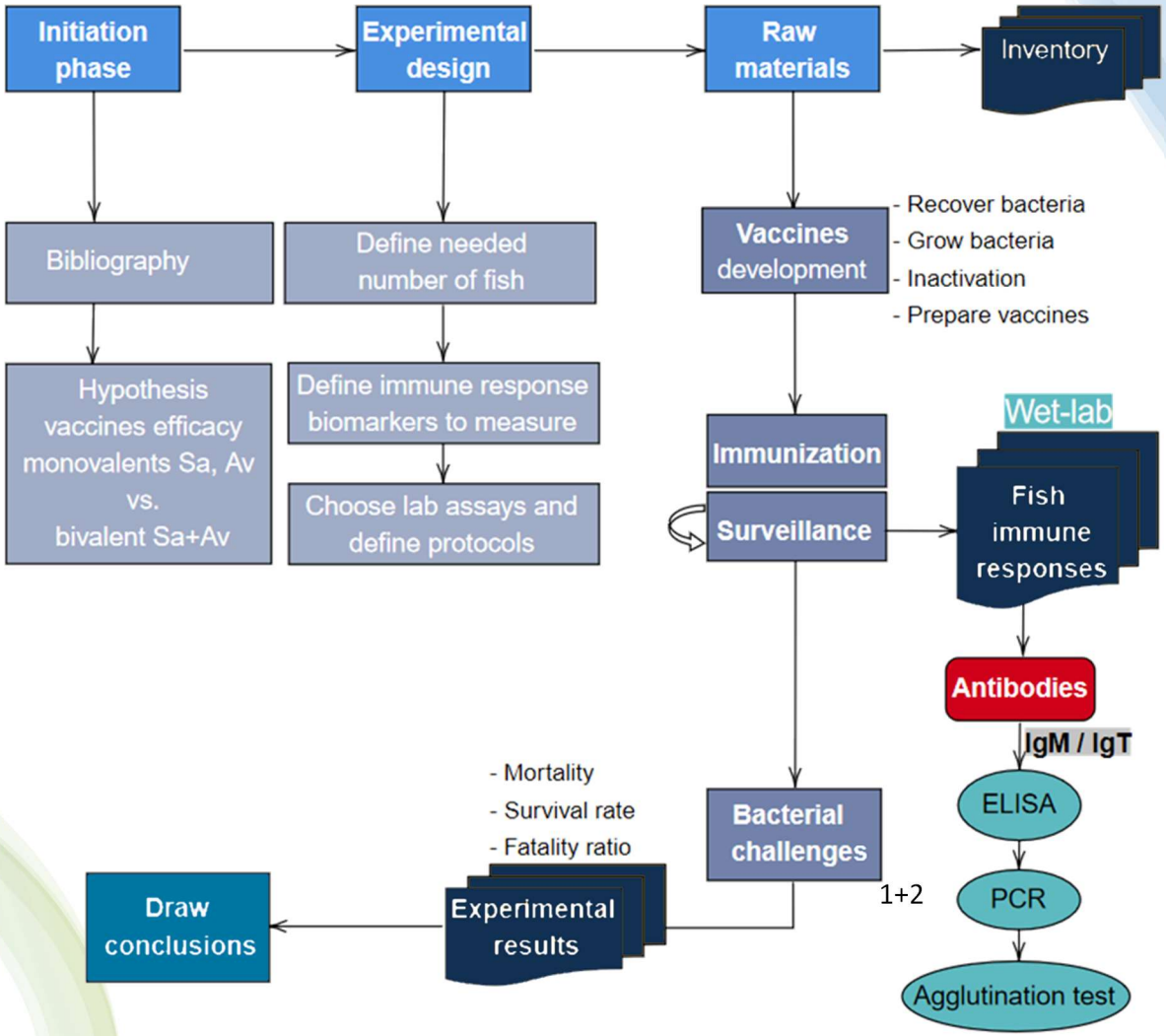
September 2021

# 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

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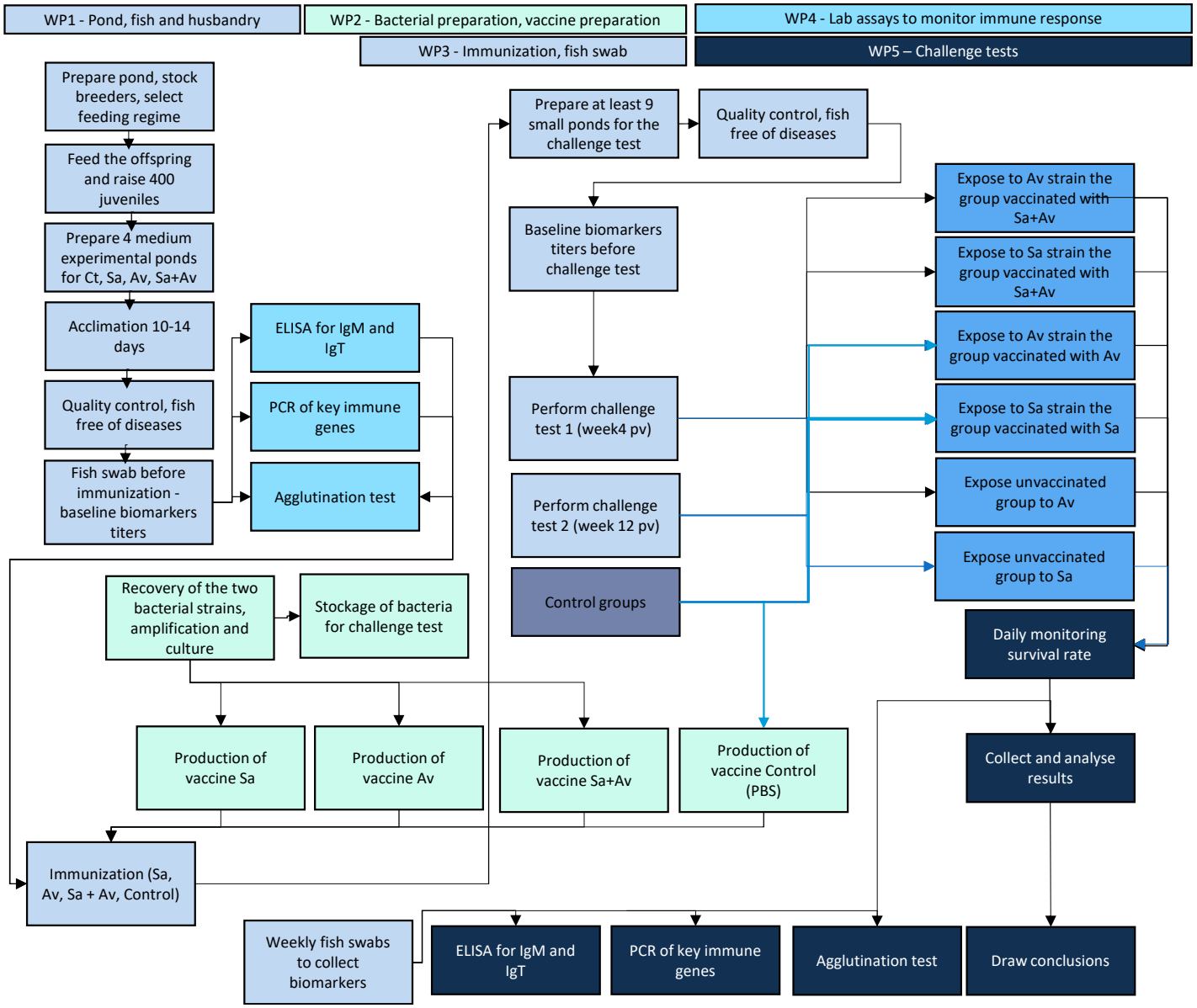
# Workflow and work packages




Experimental design ->

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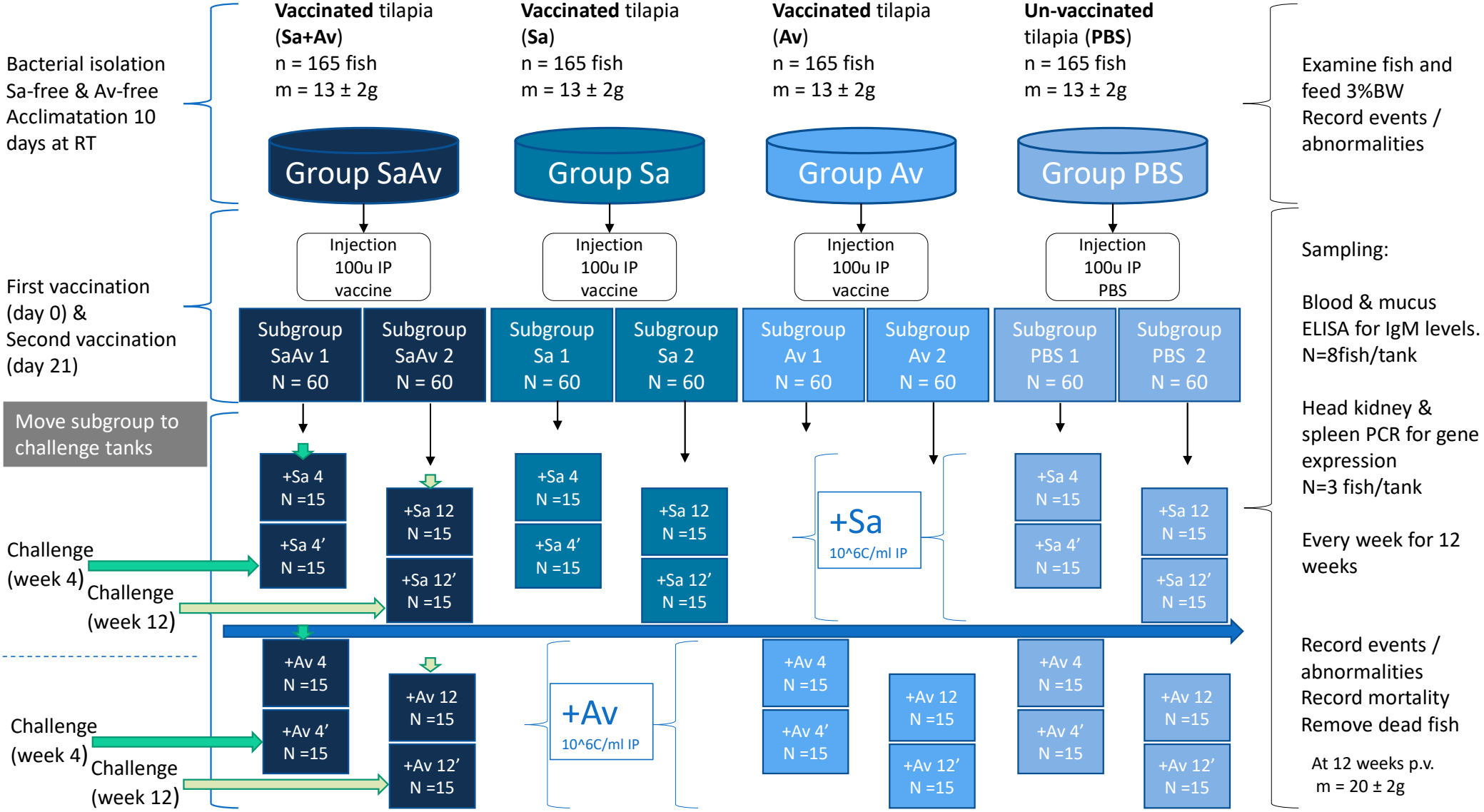

# Project Planning Timeline

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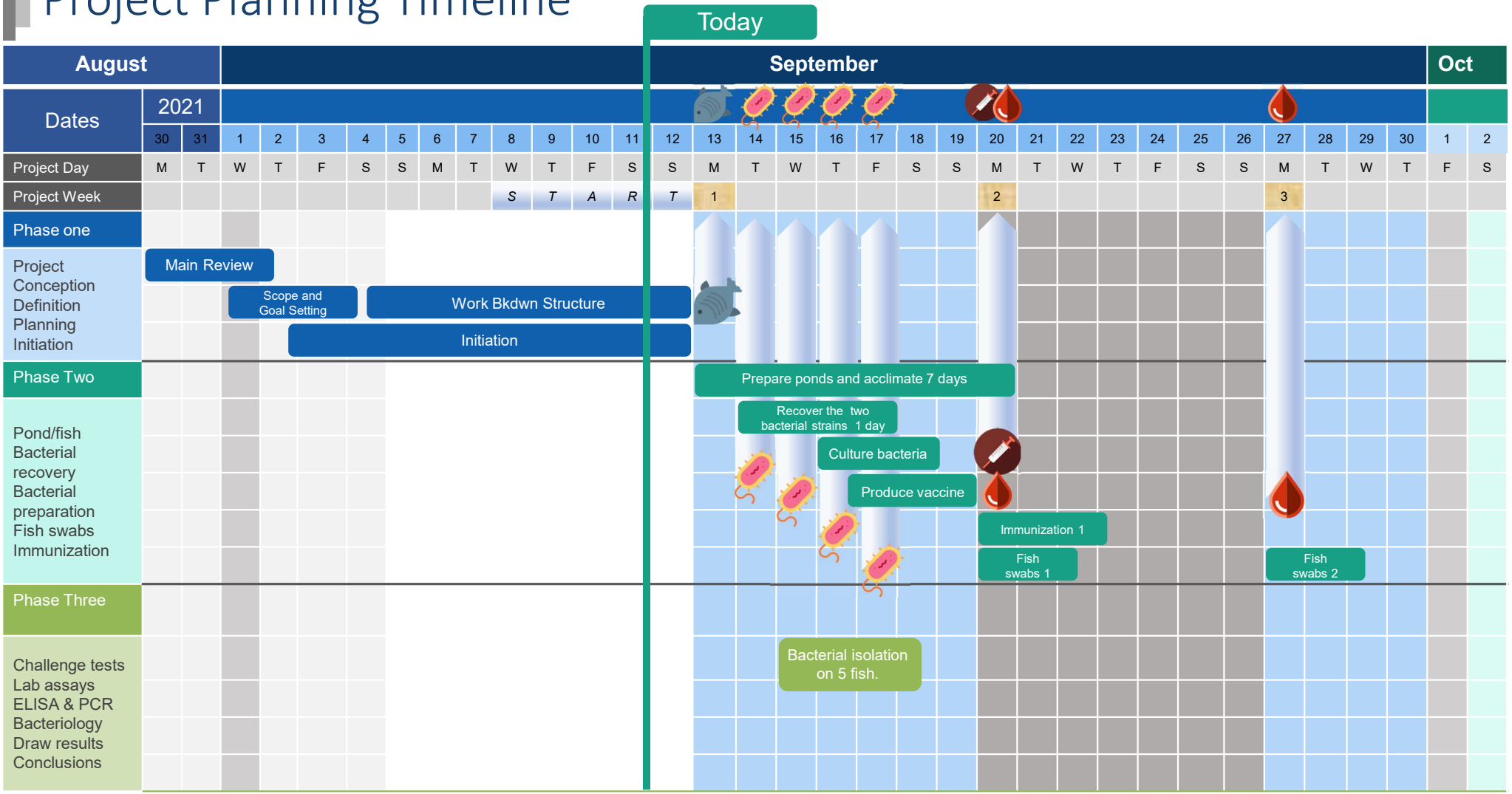

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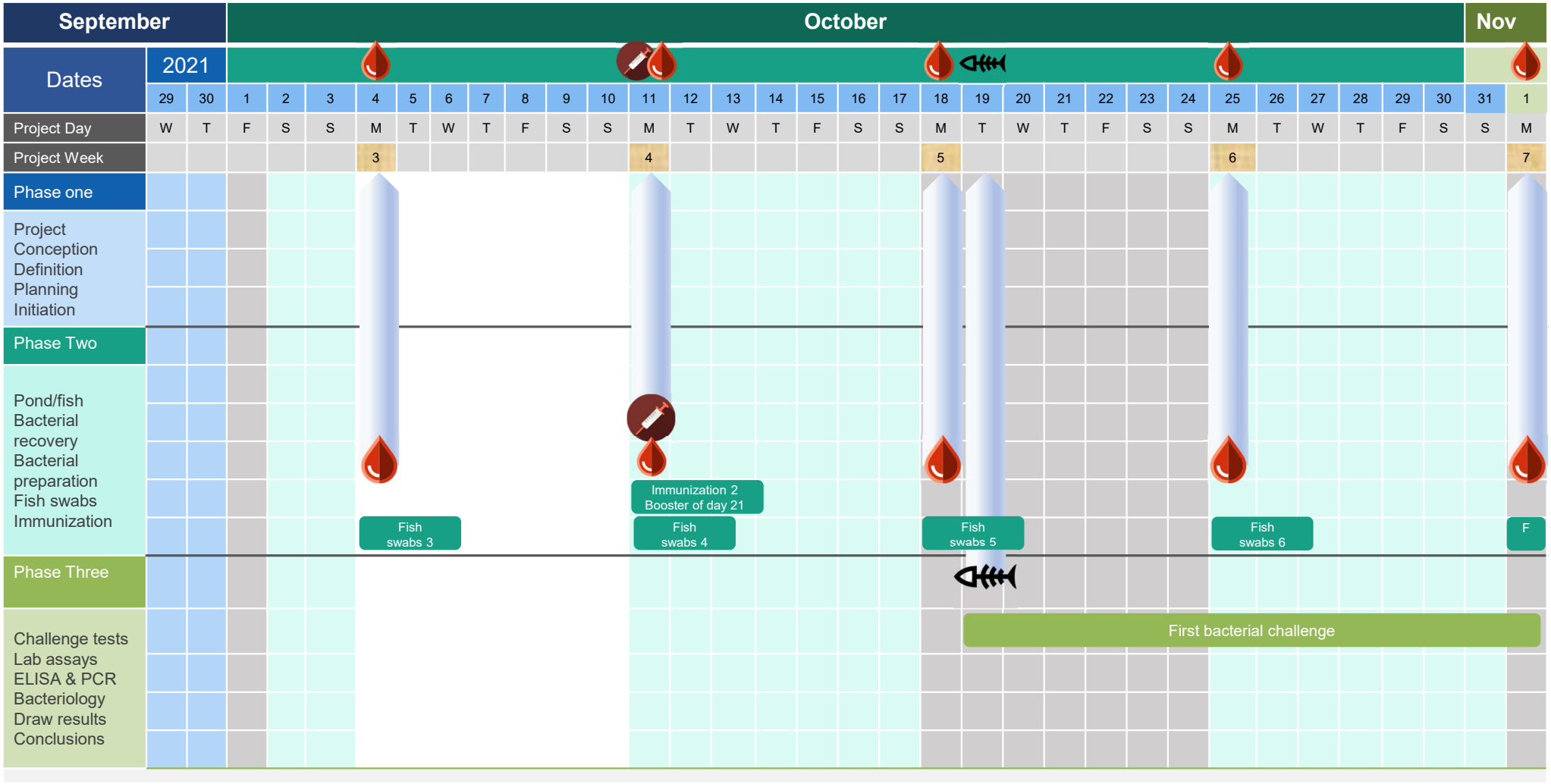

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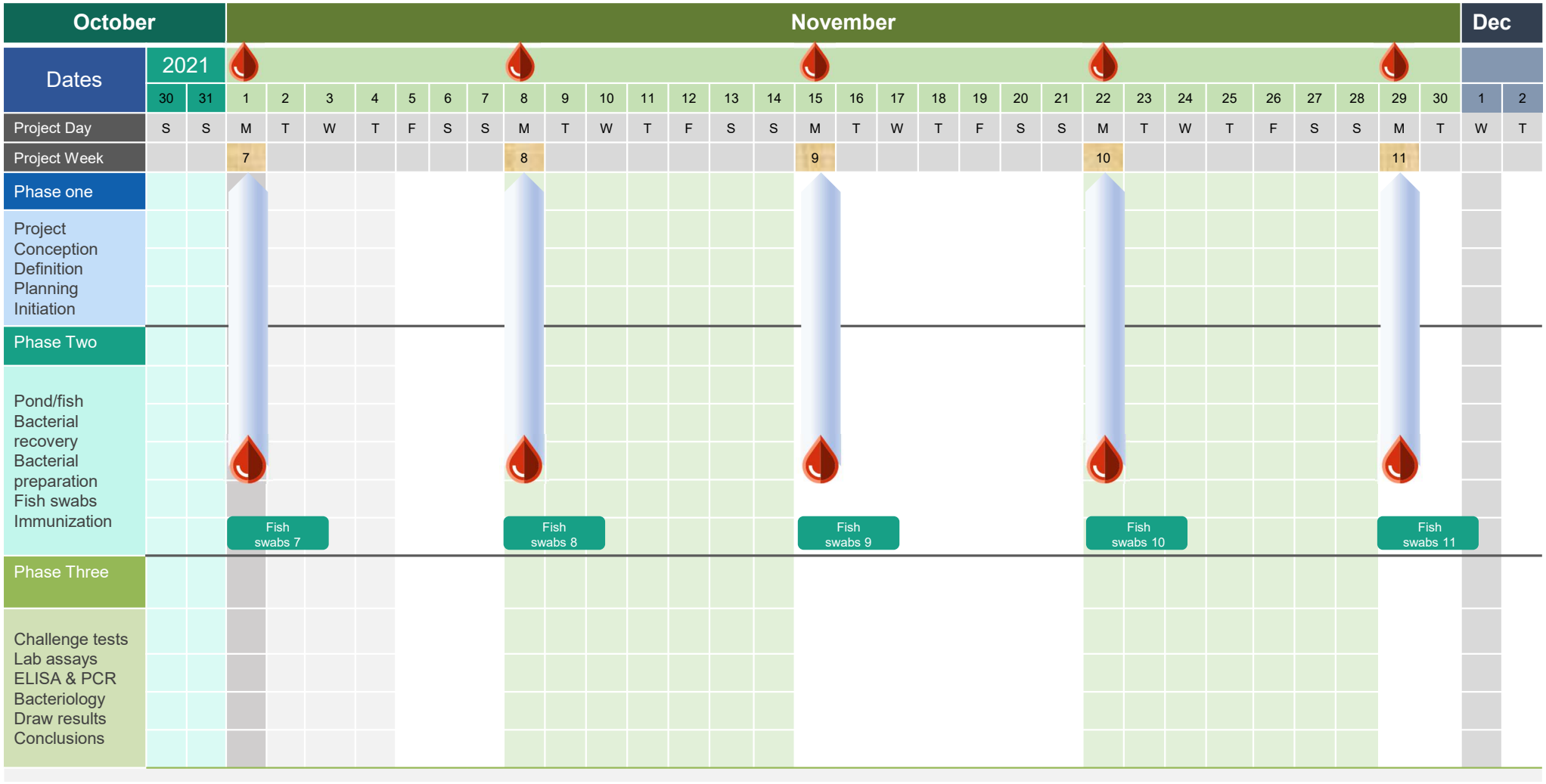

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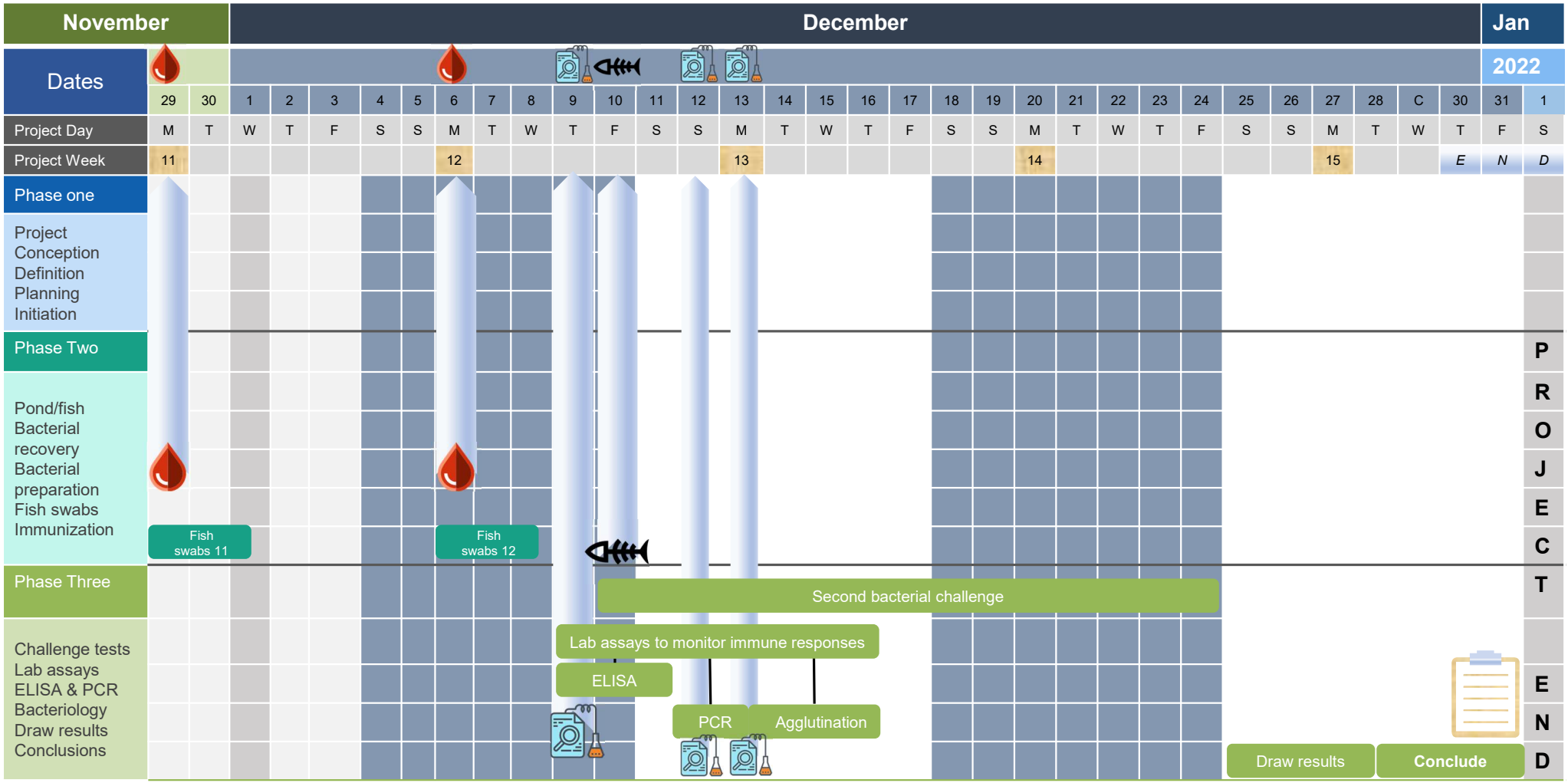

# Thank you !

# Project timeline

## Prepare aquaria, stock fish, acclimate 10 days

T1.1 Prepare the 4 aquaria.
T1.2 Assess population distribution parameters: calculate mean for mass, size and associated standard deviations (if the population is gaussian).
T1.3 Stock 165 juveniles fish of at least 12-13 grams per aquarium.
T1.4 Record water parameter.
T1.5 Define feeding regime.
T1.6 Acclimate 10 days.

Monday, September 13

Read more

## Recovery of the two strains S.agalactiae and A.veronii using TSA/TSB for 24h

T2.1 Recovery of the two strains

Wednesday, September 15

Read more

## Cultivate bacteria 24h, draw growth-curves, count viable and dead cells, estimate biomass with spectrophotometer for λ=600 nm

T2.2 Stockage of bacteria for challenge test
T2.3 Production of vaccine Sa
T2.4 Production of vaccine Av
T2.5 Production of vaccine Sa + Av
T2.6 Production of vaccine Control

Thrusday, September 16

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## Bacterial isolation for different organs in 5 randomly sampled juveniles using TSA/TSB or BHIA

T1.7 Bacterial isolation: Quality control step to prove that the fish population is free of diseases

Saturday, September 18

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## Mucus and blood swab for baseline week 0, end of acclimatation phase

T3.1 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, September 20

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## Immunization

T3.2 Immunization (Sa , Av , Sa + Av , Control )

Monday, September 20

Read more

## Mucus and blood swab for week 1

T3.3 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, September 27

Read more

## Mucus and blood swab for week 2

T3.4 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 4

Read more

## Mucus and blood swab for week 3

T3.5 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 11

Read more

## Immunization 2: booster

T3.6 Immunization second dose: booster(Sa , Av , Sa + Av , Control )

Monday, October 11

Read more

## Mucus and blood swab for week 4

T3.7 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 18

Read more

## Bacterial challenge test 1 (no booster)

T5.1 Expose to Steptoccocus agalactiae the group vaccinated with Sa
T5.2 Expose to PBS the group vaccinated with Sa
T5.3 Expose to Aeromonas veronii the group vaccinated with Av
T5.4 Expose to PBS the group vaccinated with Av
T5.5 Expose to Steptoccocus agalactiae the group vaccinated with Sa + Av
T5.6 Expose to Aeromonas veronii the group vaccinated with Sa + Av
T5.7 Expose to PBS the group vaccinated with Sa + Av
T5.8 Draw conclusions pt.1

Tuesday, October 19

Read more

## Mucus and blood swab for week 5

T3.8 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 25

Read more

## Mucus and blood swab for week 6

T3.9 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 1

Read more

## Mucus and blood swab for week 7

T3.10 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 8

Read more

## Mucus and blood swab for week 8

T3.11 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 15

Read more

## Mucus and blood swab for week 9

T3.12 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 22

Read more

## Mucus and blood swab for week 10

T3.13 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 29

Read more

## Mucus and blood swab for week 11

T3.14 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, December 6

Read more

## Lab assays to monitor immune response

T4.1 ELISA for each group of samples for IgM and IgT
T4.2 PCR of key immune genes
T4.3 Agglutination test
T4.4 Collect and analyze results
T4.5 Draw conclusions

Wednesday, December 8

Read more

## Bacterial challenge test 2

T5.9 Expose to Steptoccocus agalactiae the group vaccinated with Sa
T5.10 Expose to PBS the group vaccinated with Sa
T5.11 Expose to Aeromonas veronii the group vaccinated with Av
T5.12 Expose to PBS the group vaccinated with Av
T5.13 Expose to Steptoccocus agalactiae the group vaccinated with Sa + Av
T5.14 Expose to Aeromonas veronii the group vaccinated with Sa + Av
T5.15 Expose to PBS the group vaccinated with Sa + Av
T5.16 Draw conclusions

Thrusday, December 9

Read more

## Final phase: draw conclusions

This is the content of the last section

Friday, December 10

Read more