



**ParaFishControl**

# Vaccine for *Amyloodinium ocellatum*

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ParaFishControl Workshop

“Mediterranean Fish Parasite Management Strategies”

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# Challenge and Impact

Taxonomy NCBI

**Phylum Dinoflagellata (now Alveolata)**

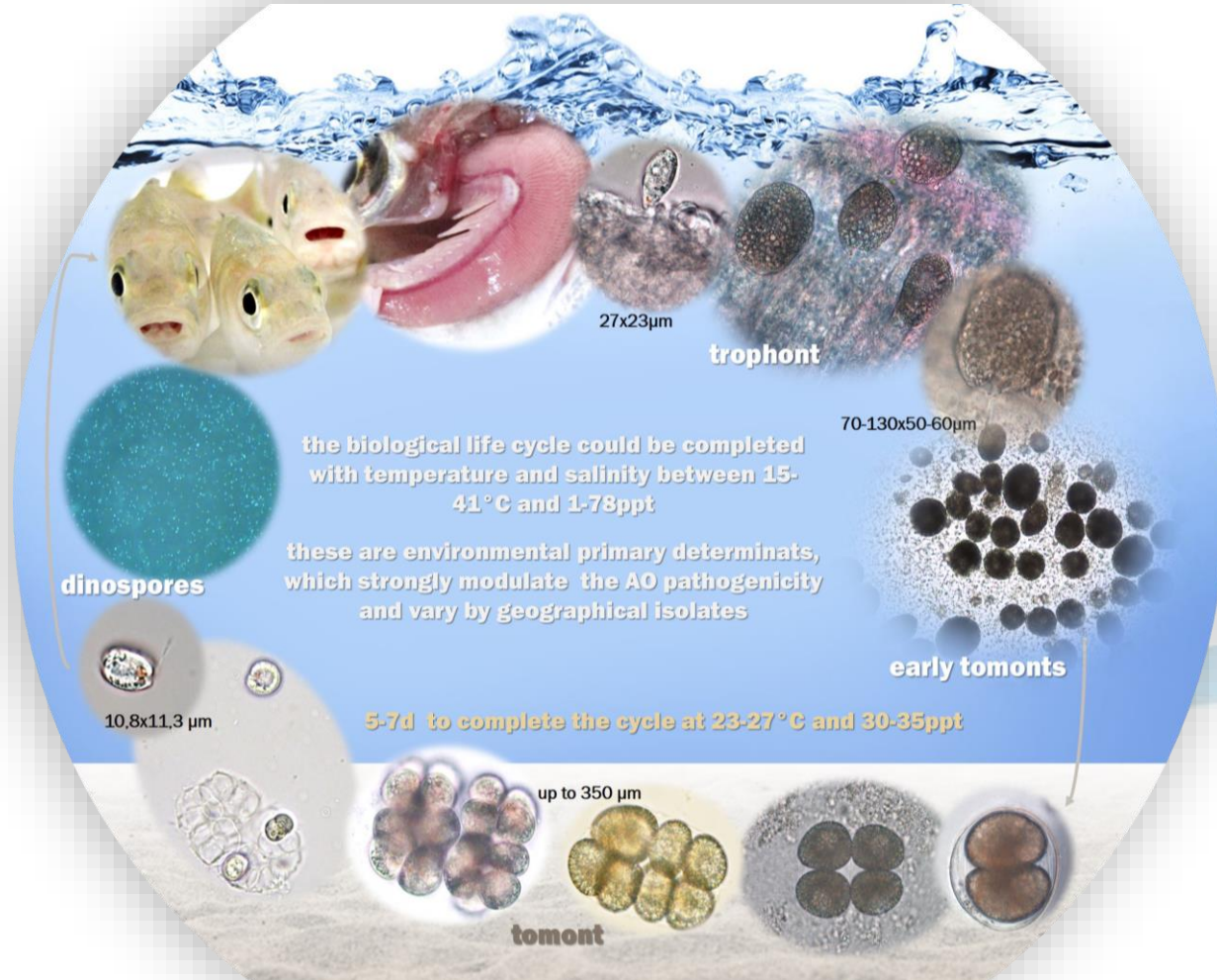
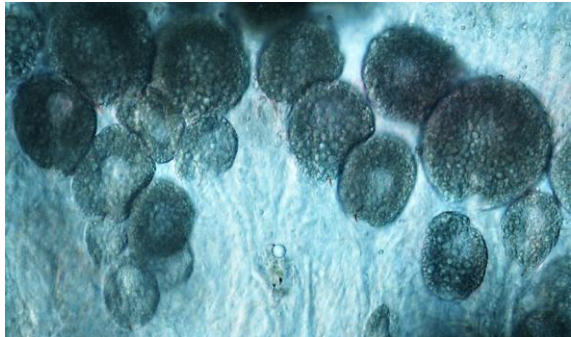
class	<i>Dinophyceae</i>
order	<i>Peridinales</i>
family	<i>Thoracosphaeraceae</i>
genus	<i>Amyloodinium</i>
species	<i>A. ocellatum</i>



*A. ocellatum* is worldwide distributed and well adaptable in tropical and temperate regions in both marine and brackish water

### low host-fish species specificity

AO infects potentially organisms belonging to 4 different phyla [Chordata, Arthropoda, Mollusca and Platyhelminthes], involving over 100 fish species belonging to 60 families



# Challenge and Impact



*A. ocellatum* (AO) is a major threat for semi-intensive/intensive aquaculture (valliculture or lagoon-based rearing sites), most of all in Southern Europe because it can cause high mortality rates, most of all in warmer months.

the mortality rate for ESB can be of 100% both in juveniles and adults.

[for example, in Northeast Italy farm two amyloodiniasis outbreaks in 2017: earthen pond with 45000 adults (weight 350 g), August, 100% mortality (markets price to the farmer 6.5€/kg, estimated losses in € 102.000 about); in the same way pond with 80.000 juveniles (weight 30g), summer, 100%]



# Our approach and our team

Brief description of the proposed solution

**INVESTIGATE ON VACCINE DEVELOPMENT**  
**crude inactivated vaccine**  
**reverse vaccinology**

Team involved in the development

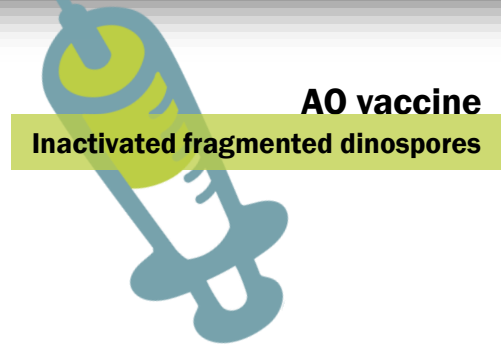


# Our proposed solution

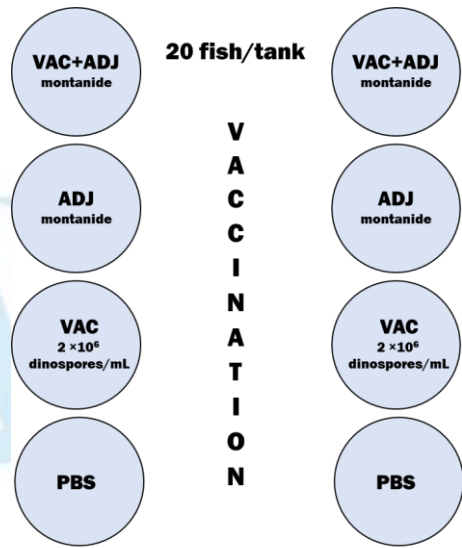
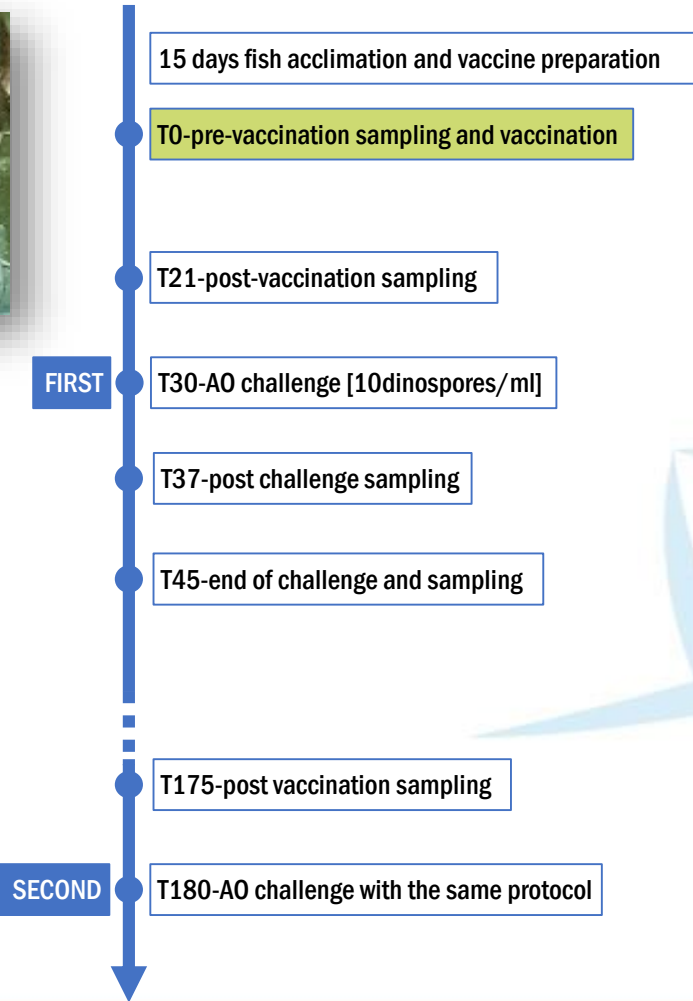


## Investigation on amyloodiniosis vaccine efficacy in European seabass (*D. labrax*)

### Experimental flow-chart

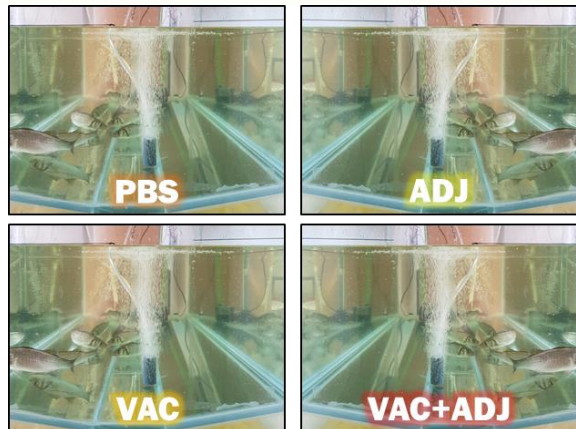


**AO vaccine**  
Inactivated fragmented dinospores

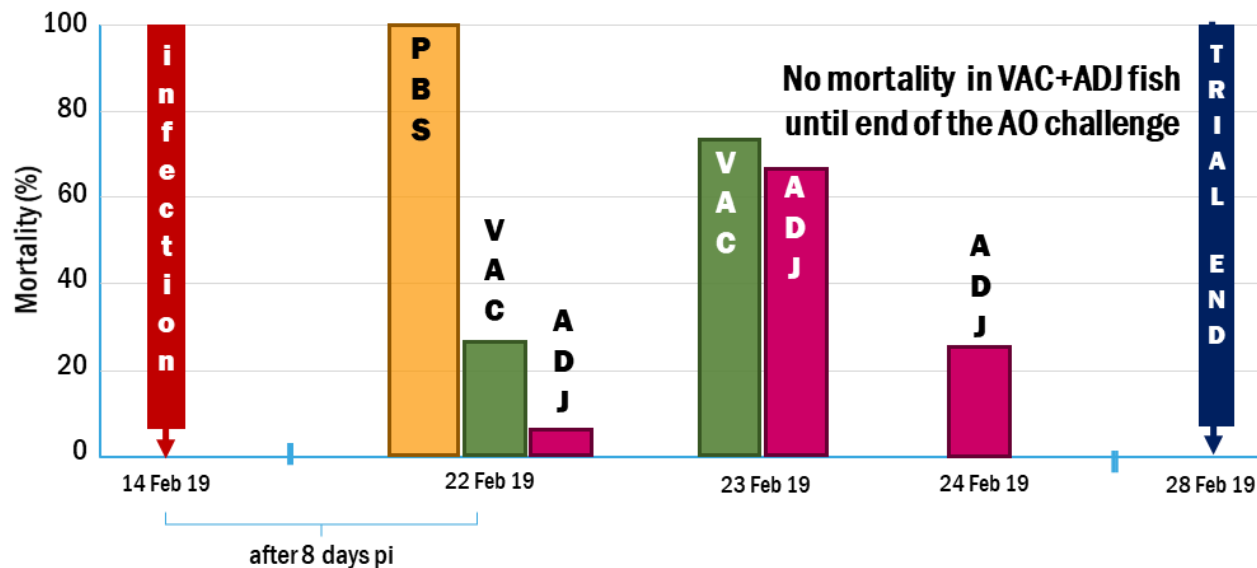


**RECIRCULATION SYSTEM**  
25-28‰  
Temp 20-21 °C

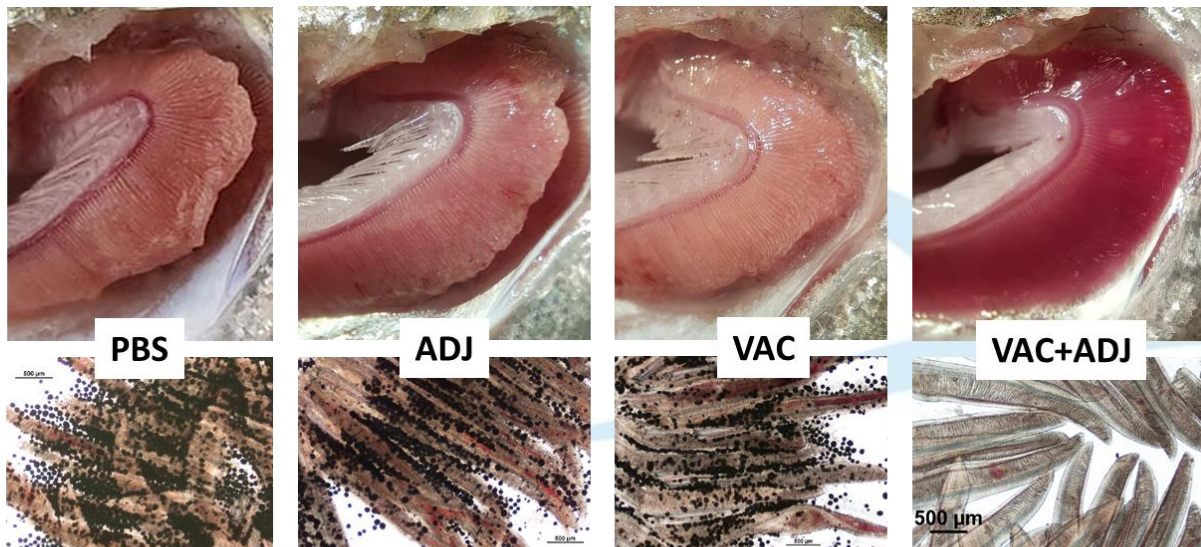




15 fish/aquarium (200L)  
salinity 26‰  
temperature 20-23°C  
infective AO dose 10 dinospore /ml



**First challenge  
30days after vaccination**



AO burden count  
mean of 3 fish/group

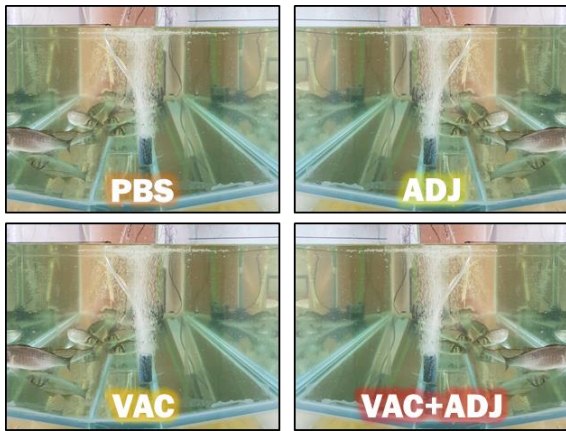
15,3×10<sup>4</sup>a/ml

15,8×10<sup>4</sup>a/ml

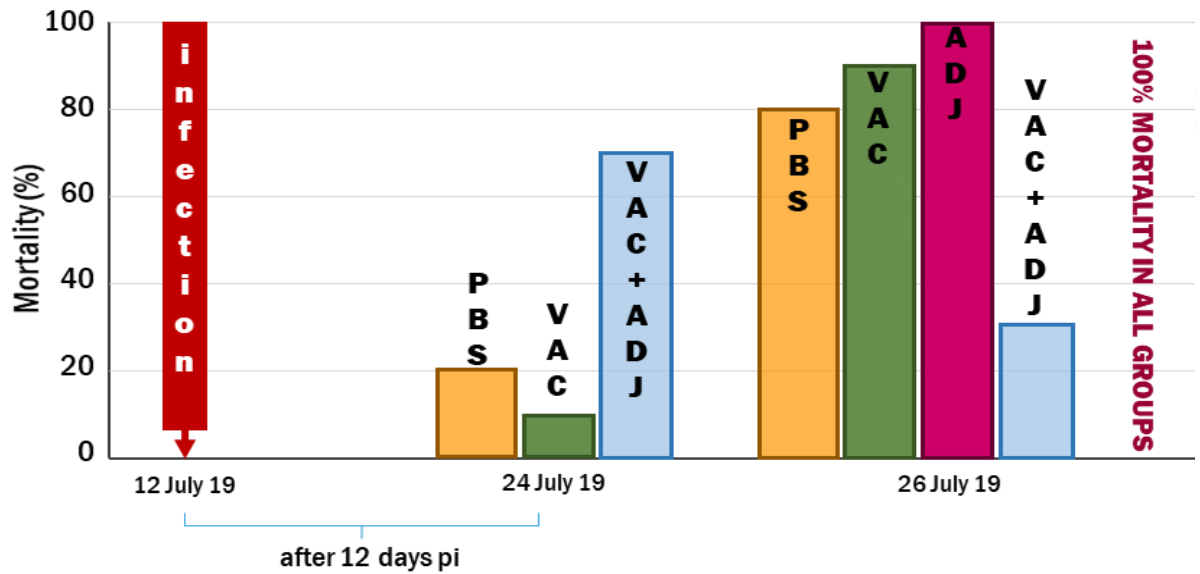
11,4×10<sup>4</sup>a/ml

0,14×10<sup>4</sup>b/ml

The AO burden is expressed as AO number/ml and is obtained using a cell chamber (S50 Sedgewick Rafter Cell) from 2 gill arches of three fish/group.

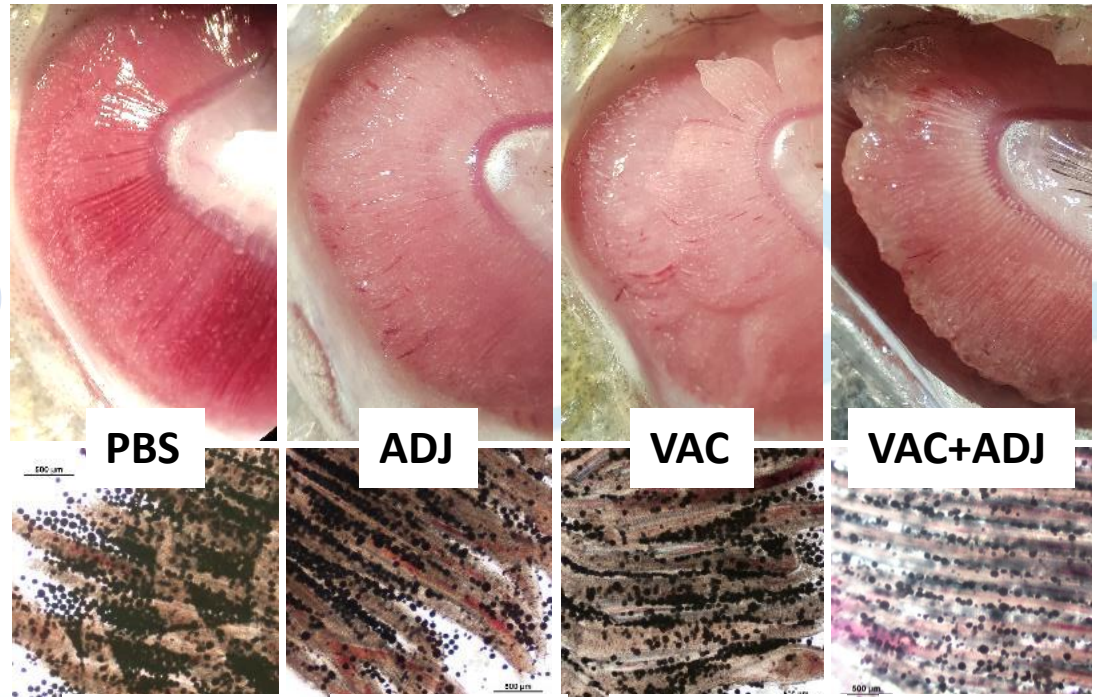


15 fish/aquarium (200L)  
 salinity 25-28‰  
 temperature 20-25°C  
 infective AO dose 10 dinospore/ml



## second challenge 6 months after vaccination

What a pity,  
 I could get sick!  
 But their work is in progress...  
 may be in future!



10,4x10<sup>4</sup>a/ml

14,3x10<sup>4</sup>a/ml

10,8x10<sup>4</sup>a/ml

5,1x10<sup>4</sup>b/ml

**future prevention of amyloidinosis and impact limitation in terms of mortality in lagoon-/land-based rearing sites**

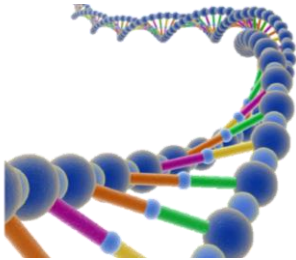
**eliminate non-legal treatments**



# Current status and next steps



intra-coelomatic injection of vaccine formulation with inactivated AO dinospores (iAOd) combined with adjuvant (Montanide ISA 763 A VG) protected ESB 30 days post-vaccination but failed to protect long term (6-month post-vaccination)



assembled *de-novo* transcriptome of *Amyloodinium ocellatum* tomons revealed several potential virulent proteins belonging to adhesin, invasion and heat shock proteins



immersion vaccination trial with inactivated AO dinospores (iAOd) combined with adjuvant Montanide IMS 1312

virulent proteins will be analysed further based on the DEG with AO dinospores to determine the most expressed

# Conclusions

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There is still a lot of work to be done but the results are encouraging to define a prophylactic strategy useful for ESB rearing in lagoon/land sites, to avoid fish losses and pollution due to the chemical treatments

Our future intent is to transfer this knowledge also to gilthead sea bream

Further insights will be orally presented by dr Byadgi (211-0)



# Thank You



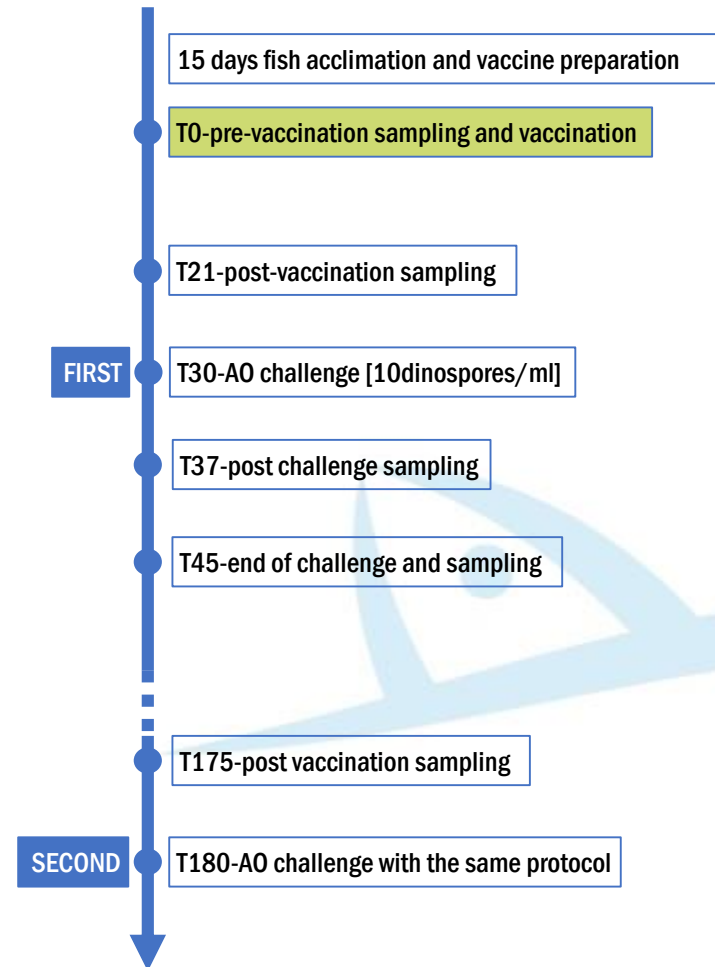
## ParaFishControl

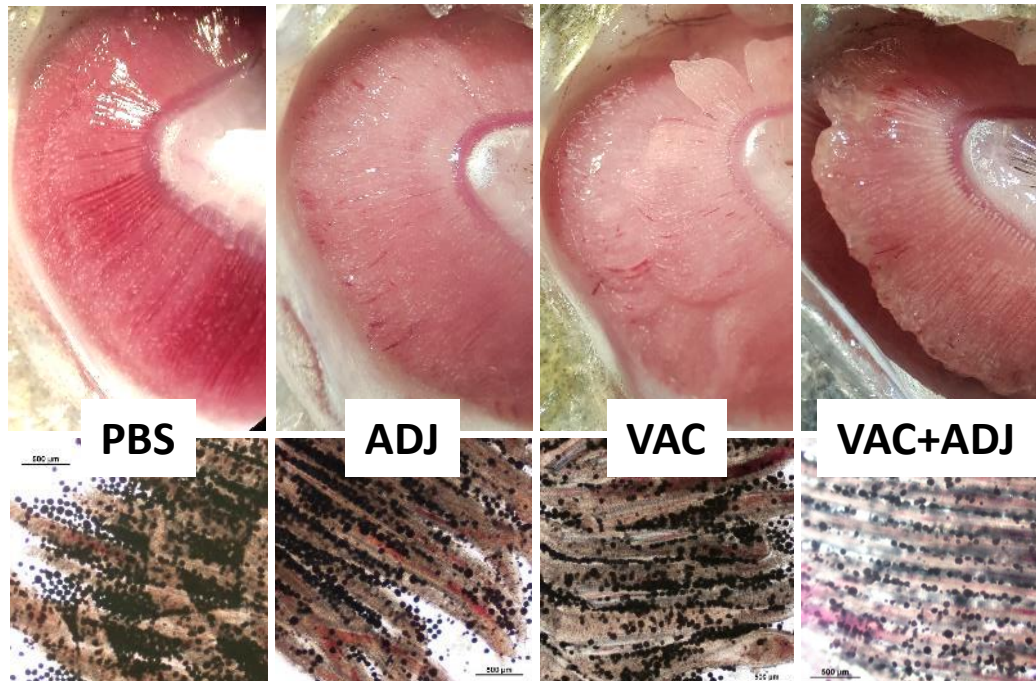
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## Experimental flow-chart





The AO burden is expressed as AO number/ml and is obtained using a cell chamber (S50 Sedgewick Rafter Cell) from 2 gill arches of three fish/group.

