Study of the ontogeny of the orbicular batfish (*Platax orbicularis*)

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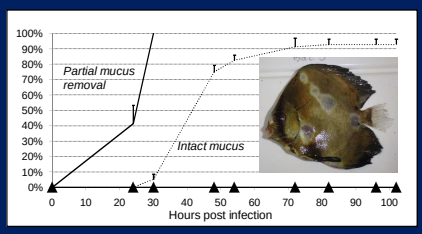
Abstract

The emerging orbicular batfish *Platax orbicularis* aquaculture industry in Tahiti is now suffering severe mortality episodes due to two bacterial agents acting simultaneously: *Tenacibaculum maritimum* and *Vibrio harveyi*. Both bacterial diseases appear shortly after the transfer of healthy fingerlings from the indoor biosecured hatchery to the offshore net-cages in Tahiti lagoon. In this context, there is an urgent need to develop strategies to control these diseases. For the first time in this fish species, a transcriptomic approach is developed to identify potential biomarkers for resistance, specific to these bacterial infections on *Platax orbicularis* by using a standardized experimental bacterial infection and a non invasive route of infection. This original approach also includes i) to describe the morpho-anatomical larval development by microscopic observations and histology, and ii) to study the expression of some targeted immune genes during fish ontogeny in relation to environmental parameters, qualifying the bacterial content of rearing water as well as other rearing compartments by a qualitative and quantitative approach. Fish were sampled every day from hatching (24 hour post fertilization) to 10 day post hatch (dph) to describe the morphological development and specifically the digestive system under referential larval rearing conditions. Gene expression of some immune-candidate targeted genes was quantified by reverse transcriptase quantitative PCR (RT-qPCR). Finally, filtrated sea water (45 µm) was sampled every day from rearing tanks, and regularly from live preys and upstream water storage tank. Bacterial concentrations were determined by qPCR targeting total bacteria, *Vibrionacea*, *T. maritimum* and *V. harveyi*. This multidisciplinary study will allow deepening our knowledge on the ontogeny of *P. orbicularis* in order to improve fingerlings survival potential and resistance to bacterial pathogens.

Experimental infection of *Platax orbicularis* juveniles with *Tenacibaculum maritimum* and a gene candidate approach

Figure 1. Cumulative mortality of *Platax orbicularis* juveniles (10 g) with intact mucus or partial mucus removal

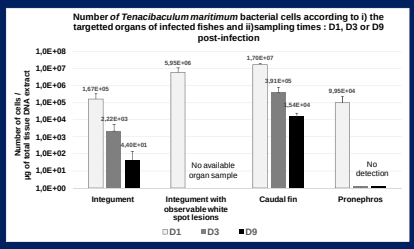


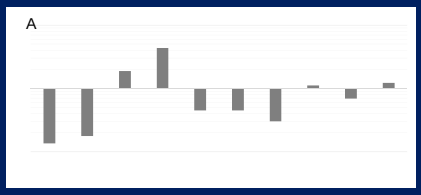
Standardized protocols of non-invasive experimental infections in controlled environment have been associated to a diagnostic test detecting the virulent bacteria *Tenacibaculum maritimum* responsible of massive mortality of *Platax orbicularis* juveniles. The fulgurate mortality rate was even increased when fish mucus was partially removed with a wet sponge (Fig.1).

Tenacibaculum maritimum was detected in all tissues analyzed, and decreased over time within these tissues (Fig. 2).

Caudal fin and the integument presenting white spots lesions were the most charged 24 h post infection. Bacterial load decreased over time on external tissues (integument and caudal fin). Head kidney (pronephros, internal organ) was contaminated at day 1, but no bacteria was detected in the next sampling days.

Figure 2. Number of *Tenacibaculum maritimum* bacterial cells according to i) the targeted organs of infected fishes and ii) sampling times : D1, D3 or D9 post-infection





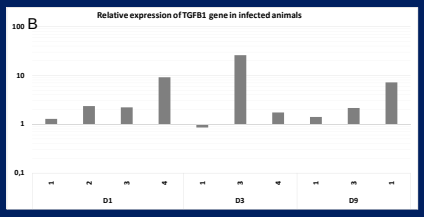


Figure 3. Expression levels of two immunity genes (A. lysozyme G and B. TGFβ1) examined as candidate bio-markers for each tissue (1: Integument, 2: Integument with white spot lesions, 3: caudal fin, and 4: Pronephros) at different sampling times (D1, D3 and D9 post infection).

Globally, gene expression levels of lysozyme G and TGFβ1 were correlated to infectious stage of *Platax* juveniles examined (n=1 per day and per condition infected or control). Lysozyme G expression level had a tendency to decrease in infected fish, while TGFβ1 was over expressed when the fish were infected.
The expression levels of immune genes within each tissue were not related to bacteria concentration.

Larval rearing protocols, bacteria concentrations and histological development of *Platax orbicularis* larvae

Figure 4. Physico-chemical parameter during the larval rearing, weaning and grow out of *Platax orbicularis*

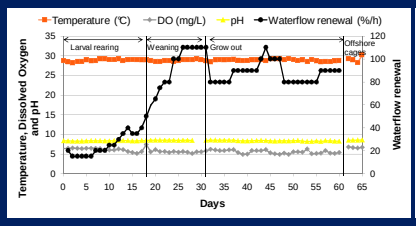


Figure 5. Number of total bacteria (entire flora) cells during larval rearing in live preys

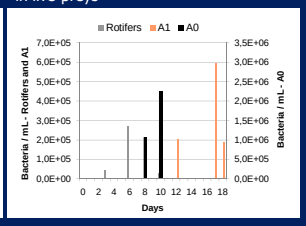
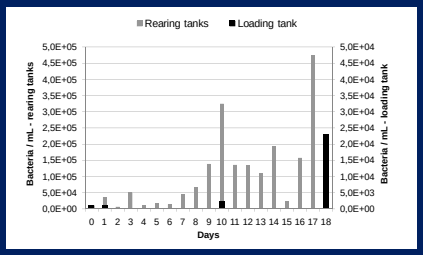


Figure 6. Number of total bacteria (entire flora) cells during larval rearing in rearing tanks and loading tank



Despite an increase in waterflow during the larval rearing and weaning periods (Fig. 4), the number of the total bacteria cells increased over time in all the compartments of the open water system (in live preys Fig.5, tanks Fig.6). No Tenacibaculum bacterial cell was detected all along the survey due to biosecurity treatments.

Figure 7. Internal development of *Platax orbicularis* larvae at hatching, day 2, 4 and 6.

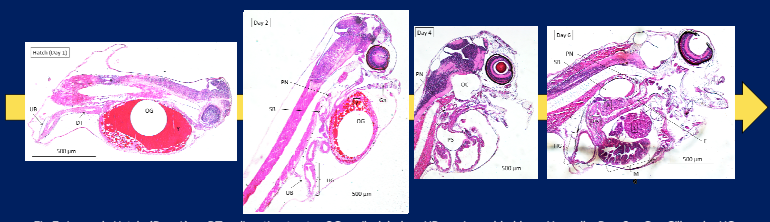


Fig.7. Legend. Hatch (Day 1) = DT : digestive tract ; OG : oil globule ; UB : urinary bladder ; Y : yolk; Day 2= Ga: Gill arch; HG: Hindgut; OG; PN: pronephros, SB: swimbladder; UB; Y; Day 4= OC: otic cavity; PN; PS: pseudo-stomach; Day 6 = E: Esophagus (with Goblet cells) ; HG ; L: Liver; MG: Midgut; P: Pancreatic tissue; PN; PS; SB;

Newly hatched *Platax orbicularis* larvae had a rudimentary digestive tract with a single gut and no major digestive organs (Fig.7 Day 1). At 2 dph, the digestive tract forms a loop, the mouth was open and the gut had differentiated into foregut, midgut and hindgut with trace of endogenous feeding material (Fig.7 Day 2. At day 4, pseudo-stomach formed with pyloric sphincter and ileo-caecal valve visibles. Gastric glands developed in the cardiac stomach of the largest 6 days old fish (Fig.7 Day 6). Notochord flexion was observed on 9 days old larvae, and metamorphosis took place between 11 and 13 days post hatch (no picture).

Conclusions and Perspectives

- After experimental infection, *Tenacibaculum maritimum* was present in all tissues analyzed, and particularly in external tissues (integument and caudal fin vs pronephros);
- Expression levels of immunity genes (Lysozyme G and TGFβ1) were modulated in response to *Tenacibaculum* infection;
- In standard larval rearing protocols, bacterial flora increased over time despite the waterflow increasing;
- Organ development was extremly rapid in *Platax orbicularis* larve raised at 29°C, with larval metamorphosis occuring around day 12 ;

- Next research will focus on the identification of bio-markers genes linked to Tenacibaculum resistance by a global transcriptomic approach (RNA-seq) , that will be secondly used to characterize gene expression profiles during larval development

- The environmental influence of microbial populations, feeding regimes and physico-chemical parameters on the biomarkers expression levels will be evaluated .