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 larval 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, filtered sea water (45 μm) was sampled every day from rearing tanks, and regularly from the prawns and upstream water storage tank. Bacterial concentrations were determined by qPCR targeting total bacteria, Vibrio harveyi, P. maritimum and V. harveyi. This multi-disciplinary 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 perital mucous removal

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 changed 24 h post infection. Bacterial load decreased over time on infected fish (Fig. 2). Head kidney (proximal organ, internal organ) was contaminated at day 1, but no bacteria was detected in the next sampling days.

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

Standardized protocols of non-invasive experimental infections in controlled environment have been associated to a diagnostic test based on the virulence 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).

Figure 3. Expression levels of two immunity genes (A, lysozyme G and B, TGFβ1) examined as candidate biomarkers for each condition. Infection 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.

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

Despite an increase in water flow 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 (live prawns Fig. 5, tanks Fig. 6). No Tenacibaculum maritimum cell was detected all along the survey due to biosecurity treatments.

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

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

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

Conclusions and Perspectives

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

5. 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.

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