



6. GENERAL CONCLUSIONS

Knowledge of the composition and dynamics of the host's gastro-intestinal microbiota, as well as from the role and mode of action of the said microbiota, and its interactions with the host's organism, are essential to develop effective solutions of disease control for the aquaculture industry.

In this respect, gnotobiotic fish, *Artemia* and *Brachionus*, can be excellent test models, to better understand the mechanisms involved in host-microbe relationships at larviculture systems, and specially to evaluate the mode of action of pre-selected probiotic strains, along the food chain.

Efforts were made to develop a standardized gnotobiotic sea bass larvae culture system, which would provide the essential framework conditions, enabling further strain testing. This research study provided valuable insights, that the system being presently developed, not only facilitates the adjustment of the most important variables (e.g. temperature, water motion, oxygen supply, etc.), but also allows the hatching of axenic eggs and the efficient maintenance of sea bass larvae under gnotobiotic conditions, for an additional 13 day period.

Both the disinfectant concentration and the development stage at which the antimicrobial treatments were applied, seemed to have no adverse influence either on the hatchability of the exposed sea bass eggs, or in the larvae performance.

The results indicate that surface disinfection treatment with 100 mg.L⁻¹ glutaraldehyde for 5 min, and further antibiotics supplements (ampicillin and rifampicin, 10 mg.L⁻¹ each), have proven to be highly efficient in reducing microbial load and enhancing early larval survival, for all set-up groups under evaluation. Moreover, the sensitivity of the method appeared to be fairly correlated with the egg quality and microbial load prevalence. Although biocenosis influences played a significant part on hatching success, variations in the chorion thickness and in egg quality, may explain some of the differences with replicates from different egg-batches, along the seasonal spawnings. For that same reason, an agreement with the supplier of sea bass eggs, on a standard operational routine, would be beneficial.

Moreover, to confirm the real efficacy of the disinfection treatments employed and the consistency of its results, so that its use shall become standard practice, further evaluation should be undertaken. Nonetheless, complementary studies (e.g. histological and further morphological development analysis) should be carried out, in order to ensure that no long-term adverse effects associated with the treatments, would result.

Furthermore, although the development of unwanted resistant bacteria was not confirmed, the problem of eventual resistance development should not be neglected.

The success in culturing sea bass gnotobiotic larvae, also depended on the application of appropriate

experimental apparatus (bottles, multi-well plates, vials, aeration tubes, rotating devices, etc.), able to reproduce and maintain the complex environment conditions, in which experimental organisms could subsist in a gnotobiotic state.

Additionally, the use of sterile polystyrene multi-well plates, proved to be quite adequate as a reliable approach to assess the egg hatching rate.

Nevertheless, the husbandry protocols herein evaluated, still require a number of improvements. It can be certainly anticipated that an improvement on the sterilization and disinfection procedures, should be undertaken so as to prevent the prevailing microorganisms contaminations.

Furthermore, although the evaluated rotating devices imposed stress to sea bass larvae, they are probably at present, the best means to maintain bacteria or yeast cells in suspension, and should be considered in future experimental trials. Alternatively reduced rotation speeds could be tested, to try to lessen stress on larvae.

As was later on confirmed, *Aeromonas hydrophila* (at 10^5 CFU/ml), Wild Type baker's yeast and its mnn9 isogenic mutant (at 10^6 cells/ml), acted as neutral microorganisms, not influencing the larvae performance during the first thirteen days. Independently of the fact that, the results attained, fell beyond the initial expectations, these strains, which in the past have proven to be highly appropriate as performance-enhancing, should be further tested. On the other hand, consideration should be given to increase the experimental time period, so as to allow the proper settling and adherence of the microbial strains. Therefore, studies using this standardized gnotobiotic aquatic organism, should be extended to juvenile stages, the gnotobiotic food chain expanded.

In brief, it is hoped that the gnotobiotic larval growth system herein described, will be further developed so as to allow the coherent establishment of a gnotobiotic aquatic food chain to further study the host-microbiota interactions.