LIVE FOOD MICROBIAL MANAGEMENT AND ITS ROLE IN CONDITIONING THE LARVAL REARING ENVIRONMENT

Letizia Chiappi [1]*, Mirko Pierantozzi[1], Tania De Wolf[1], Giorgia Bignami[2], Geert Rombaut[3]

[1]INVE Aquaculture Research Center, Italy

[2]University of Bologna, Department of Veterinary Medical Sciences

[3] INVE Aquaculture, Belgium

l.chiappi@inveaquaculture.com

Introduction

Microbial management in hatcheries is particularly critical during the early larval stages, when larvae are highly sensitive to microbial imbalances and the tank microbiome is still developing. This instability increases the risk of microbial disruptions and opportunistic bacterial proliferation. Beyond pathogen suppression, modern microbial management strategies therefor focus on steering the early establishment of beneficial microbial communities that support water quality, enhance microbial stability and promote larval health. This study evaluated disinfection and MIC-F-based microbial management strategies during live food production (rotifers and Artemia) and assessed their impact on the larval rearing environment and seabream larval performance.

Materials and methods

Identification of Sanolife MIC-F Bacillus strains: Pure cultures of the *Bacillus* strains (*B. subtilis, B. licheniformis,* and *B. pumilus*) were plated on Tryptic Soy Agar (TSA) and incubated at 37 °C for 24 h Prior to the start of the trial, strain identity was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). .

Rotifer treatments: L-type rotifers were cultured on a diet consisting of 85% fresh baker's yeast and 15% Roboost and Vitalgae Nanno. Prior to larval feeding, rotifers were enriched with Easy Dry Selco (EDS). Following enrichment, rotifers were subjected to one of two treatments:

1. **Control treatment**: no use of Sanolife MIC-F and Sanocare SURE.
2. **Selective microbial management**: a 20-min bath applying Sanolife MIC-F at 2.5 g L^{-1} together with Sanocare SURE at 1 g L^{-1} to steer the microbiota of the rotifers

The microbial community associated with rotifers was analyzed using culture-based and molecular methods. Bacterial abundance was determined by plate counts on TSA and on TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar. The amount of *Bacillus* spp. present in the sample were estimated by plating the sample on TSA at 37 °C for 24 h. Additionally, Illumina 16S rRNA gene sequencing was used to identify dominant bacterial phyla.

Artemia treatments: Three production protocols were evaluated. In all treatments, nauplii were produced using EG SEP-Art® or EG SEP-Art® D-FENSE, followed by specific post-hatch management:

1. **Control**: Artemia produced using EG SEP-Art® without additional microbial managment
2. **SEP-Art D-FENSE–based management**: EG SEP-Art® D-FENSE cysts were hatched and transferred after 21 h to enrichment tanks, where enrichment was performed using EDS and a water conditioner.

3. **SEP-Art D-FENSE followed by microbial enhancement**: Artemia were produced as in treatment 2. After enrichment, nauplii were rinsed, concentrated to 5 million L^{-1}, and incubated for 30 min with germinated *Bacillus* (Sanolife MIC-F activated for 1 h at 30 °C in sterile 25 ppt water), followed by cold storage with 250 ppm peracetic/peroxide-based sanitizer.

As for the rotifers, identical analyses were conducted using culture-based bacterial plating and Illumina-based 16S rRNA gene sequencing to characterize associated microbial communities.

Seabream larval rearing: Larval rearing was conducted under three live-food-derived microbial management strategies:

1. **Negative control**: live food produced without microbial management.
2. **Positive control**: live food produced using microbially managed rotifers and EG SEP-Art® D-FENSE–based Artemia production.
3. **Microbial control**: live food produced under microbial management protocols combined with MIC-F microbial enhancement.

For each treatment, two replicate 6,000-L tanks were stocked with 600,000 just-hatched gilthead seabream (*Sparus aurata*) larvae. Green-water conditions were maintained during the first 30 dph by daily addition of VitAlgae Nanno (60–70 $g.m^{-3}$). Larvae were fed treated rotifers from 3 to 30 dph, AF from 16 to 26 dph, and enriched EG SEP-Art® (D-FENSE) Artemia from 22 to 53 dph. A commercial dry diet (O.Range, INVE Aquaculture, Thailand) was introduced at 19 dph and fed until weaning at 55 dph. After weaning, medium-sized larvae were selected and monitored for an additional three weeks of post-weaning.

Tank biofilm bacterial abundance was evaluated at 30 and 56 dph by swabbing 5 cm^2 of tank surface, followed by homogenization and plating.

Larval performance was assessed based on survival, growth, stress resistance, deformity incidence, and fatty acid methyl ester (FAME) profiles. At 55 dph, larvae were graded to determine fry survival and quality across treatments.

Results

No significant differences in larval growth or quality were observed among treatments, but clear differences emerged in the microbial profiles of Artemia and tank biofilms. Disinfection-based treatments produced significantly cleaner Artemia, with ~2-log reductions per nauplius in both total bacteria and *Vibrio* load. Probiotic *Bacillus* strains were effectively incorporated, averaging ~1.6×10^3 bacilli per nauplius after 4 h of cold storage in treated Artemia. In tank biofilms, *Bacillus* spp. persisted up to 30 dph when MIC-F was applied to rotifers, and up to 56 dph at higher levels (~10^5 bacilli per 0.5 cm^2) when MIC-F was supplied via both rotifers and Artemia.

Discussion

These findings support the view that microbial management in larval hatcheries contribute to environmental conditioning. By facilitating the early establishment and maintenance of beneficial microbial communities, *Bacillus*-based microbial management strategies contribute to enhanced system stability and resilience. Such advantages are likely to become most evident under suboptimal or stressful rearing conditions, where microbial balance plays a critical role in larval health.