# Towards controlled live food microbiomes: From clean live food to controlled live food in European seabass and seabream hatcheries

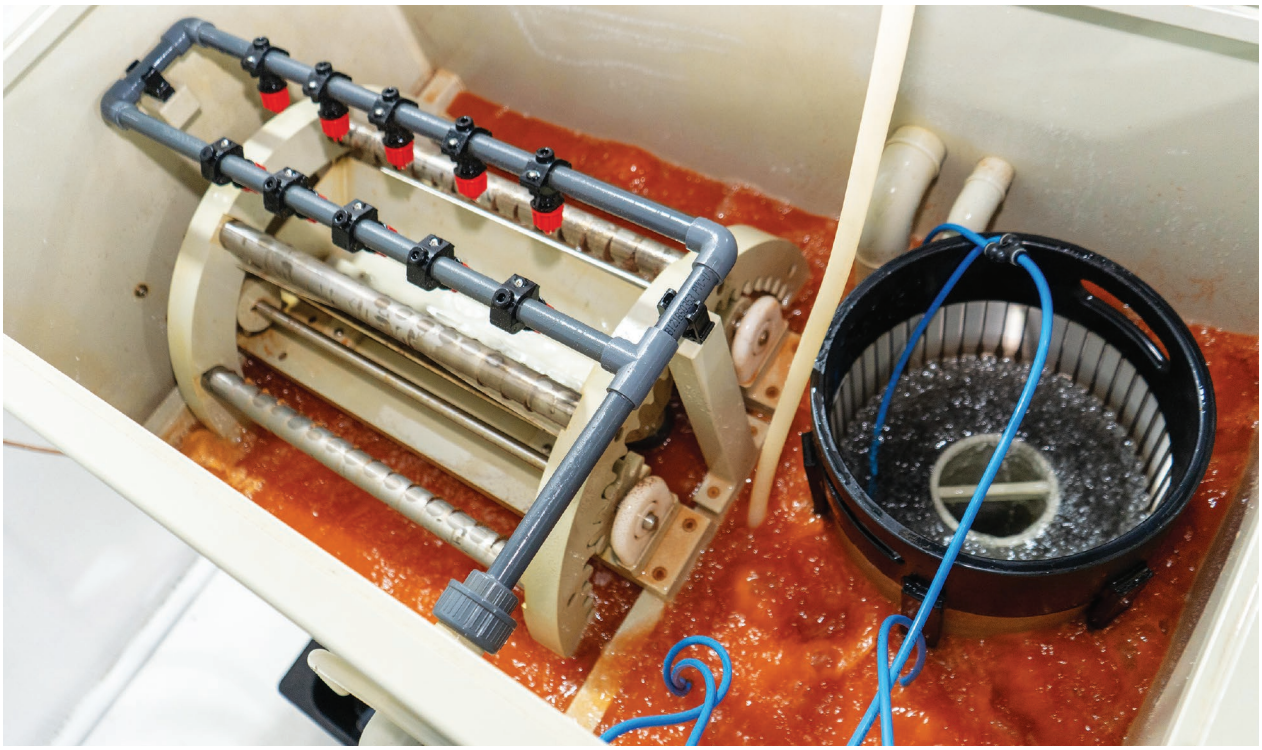
**Geert Rombaut, Tania De Wolf**
INVE Aquaculture



European seabass and gilthead seabream hatcheries have made substantial progress in live food production, larval nutrition and operational control. Yet the earliest stages of larval development remain biologically vulnerable. Larval immune and digestive functions are still developing while the microbial communities associated with the larvae and their rearing environment are also becoming established.

Hatchery biosecurity has traditionally focused on exclusion, hygiene and disinfection. These measures remain essential, but a broader approach is emerging. Rather than only limiting harmful microorganisms, modern microbial management aims to actively shape microbial communities to support animal health, rearing conditions and overall biosecurity.

Building on scientific findings generated over recent years, INVE has translated these concepts into practical protocols for commercial hatcheries. Trials conducted in rotifer and *Artemia* production and during gilthead seabream larval rearing evaluated the combination of microbial-control technologies with water conditioning using selected beneficial *Bacillus* strains. The work



investigated how these microorganisms establish in the live food production environment, become associated with the live food, and subsequently influence microbial dynamics within the larval rearing environment.

The objective is no longer simply to produce live food with fewer bacteria. It is to influence which microorganisms are associated with live food, how those communities develop and what they introduce into the larval rearing system.

### Live food quality: More than nutrition

Rotifers and *Artemia* are indispensable during the first feeding stages of European seabass and seabream. Their quality encompasses several dimensions: reliable production and availability, physical quality, nutritional value and microbial quality.

While enrichment has long been used to optimize the nutritional composition of live food, the microbial communities associated with rotifers and *Artemia* are increasingly recognized as another important factor influencing larval health and rearing success.

This makes live food an important microbial interface. It delivers not only nutrients, but also microorganisms that can influence the microbiota associated with the larvae, tank water and tank surfaces.

> *"True live food quality goes beyond nutrition alone. It is built on four pillars: reliable supply, physical quality, nutritional value and microbial quality."*
> **Geert Rombaut**

Importantly, similar total bacterial counts do not necessarily represent similar microbial quality. Two batches may contain comparable bacterial concentrations but very different microbial communities.

Without robust and consistently applied Standard Operating Procedures (SOPs), small variations during culture, enrichment, harvesting or storage can introduce microbial "noise". This variability can subsequently be transferred to the larval rearing environment, contributing to less predictable microbial dynamics and production outcomes.

Microbial management should therefore combine three complementary objectives: control undesirable bacteria, promote beneficial microorganisms and ensure consistent execution of live food protocols.

### Control the microbial starting point

*Artemia* production involves several stages, from hatching and separation to enrichment, harvesting and storage, each of which can influence the microbial quality of the final product.

Conditions established during hatching are particularly important because they define the starting point for subsequent production stages. High bacterial loads entering enrichment can increase oxygen consumption, affect water quality and create less predictable enrichment conditions.

EG SEP-*Art*® D-FENSE was developed as a first line of defense against *Vibrio* in *Artemia* production, helping hatcheries establish cleaner and more stable microbial conditions from the beginning of the process.

Trials have shown that hatching with EG SEP-*Art*® D-FENSE reduces *Vibrio* levels in hatching water by approximately five logarithmic units (99.999%) and total bacterial loads by three logarithmic units (99.9%).

The significance of these reductions goes beyond producing cleaner *Artemia* nauplii. By limiting microbial noise early in the process, hatcheries can establish more predictable enrichment conditions and create a stronger foundation for delivering live food with more stable and controlled microbial quality.

*"The microbial profile of **Artemia** should be the result of deliberate management, not chance. Controlling microbial quality from hatching onwards lays the foundation for more consistent live food."*
**Geert Rombaut**

## Extending microbial quality across the *Artemia* feeding program

The SEP-*Art*® D-FENSE portfolio is being expanded beyond EG to include AF SEP-*Art*® D-FENSE and BF SEP-*Art*® D-FENSE, bringing D-FENSE microbial quality technology to specialty *Artemia* cysts used during a critical phase of marine larviculture. AF SEP-*Art*® and BF SEP-*Art*® are typically fed during the transition from rotifers to enriched *Artemia*, a relatively short but highly sensitive period in which larvae undergo major changes and are also vulnerable to microbial challenges.

By extending D-FENSE technology across AF SEP-*Art*®, BF SEP-*Art*® and EG SEP-*Art*®, hatcheries can now apply a consistent microbial quality management strategy throughout successive *Artemia* feeding stages, while still selecting the most appropriate *Artemia* type to match the biological and nutritional requirements of the larvae. This provides greater continuity in live food quality and supports a more robust larval rearing program from the first *Artemia* feeding onwards.

## Condition the microbial environment

Reducing undesirable microorganisms can improve the microbial quality of live food and provide a more controlled starting point for subsequent production steps. However, microorganisms from the culture environment, water, equipment and handling practices can continue to influence the microbial community associated with the live food.

Selective microbial management aims to support the establishment of beneficial microbial populations following microbial reduction measures. Sanolife™ MIC-F is a water conditioner containing selected strains of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*. Applied to the live food production environment, these beneficial microorganisms colonize the water and can subsequently become associated with rotifers and *Artemia*, helping to modulate the microbiota of ready-to-feed live food.

*"Disinfection can reduce microbial pressure, but it does not determine which microorganisms recolonize the system. Selective microbial management aims to influence that next stage by conditioning the live food environment with functional bacteria under controlled conditions."*
**Tania De Wolf**

## Rotifers as microbial carriers

Rotifers are among the first live prey offered to seabass and seabream larvae and therefore represent an important link between live food production and the developing larval environment.

Different microbial management strategies combining Sanocare™ SURE, a natural *Vibrio* controller, with the water-conditioning action of Sanolife™ MIC-F were evaluated at several stages of the rotifer production process.

Sanolife™ MIC-F was applied to condition the water during rotifer culture, enrichment and post-enrichment management. Across these approaches, beneficial *Bacillus* established in the live food environment and became associated with the rotifers. Microbial community modulation included:

- reduced relative abundance of Proteobacteria, particularly *Vibrio* spp.;
- increased relative abundance of Firmicutes, including *Bacillus* spp.; and

- reduced *Vibrio* loads when Sanocare™ SURE was integrated into the protocol.

The different application routes demonstrate that microbial conditioning can be adapted to different hatchery workflows while maintaining the same biological objective: reducing microbial pressure and promoting a more controlled live food microbiota.

## Conditioning the microbial environment during *Artemia* production

Building on the microbial control provided by EG SEP-*Art*® D-FENSE, INVE evaluated different strategies for conditioning the *Artemia* production environment with beneficial bacteria while maintaining the nutritional and physical quality required for larval feeding.

The trials assessed the application of Sanolife™ MIC-F as a water conditioner at different stages of the process, including during enrichment and after enriched *Artemia* had been harvested and concentrated. In both approaches, the beneficial *Bacillus* established in the surrounding water and subsequently became associated with the *Artemia*. Across treatments, the microbial profile shifted towards a higher abundance of *Bacillus* spp., while the reduced *Vibrio* background achieved through the SEP-*Art*® D-FENSE process appeared to favor the establishment of these beneficial microorganisms.

One practical application involves the addition of activated Sanolife™ MIC-F to condition the water during cold storage of *Artemia* nauplii. During this period, the beneficial *Bacillus* establish in the surrounding environment and become associated with the *Artemia*. In INVE-led trials, approximately $1.6 \times 10^3$ bacilli per nauplius were detected after four hours of cold storage in water conditioned with Sanolife™ MIC-F, without affecting the nutritional quality of the live food.

These results demonstrate how water conditioning can be integrated into a controlled live food strategy. By first reducing undesirable microorganisms and subsequently conditioning the live food environment with selected beneficial bacteria, hatcheries can combine nutritional performance with a more deliberately managed microbial profile throughout the live feeding program.

## Extending microbial management into the larval tank

The potential influence of microbial conditioning does not end when live food leaves the production system; it continues to shape the microbial environment after entering the larval tank.

An INVE-led gilthead seabream trial evaluated whether microorganisms delivered through live food could influence the microbial environment within the larval rearing system.

No significant differences in larval growth or quality were observed under the controlled conditions evaluated. Clear differences did, however, emerge in the microbiological results.

Microbially managed *Artemia* showed approximately two-log reductions in total bacterial and *Vibrio* loads per nauplius.

More importantly, beneficial *Bacillus* delivered through live food persisted within tank biofilms. When rotifers produced in MIC-F-conditioned water were supplied to the larvae, *Bacillus* remained detectable in the tank biofilm until 30 days post-hatch. When both rotifers and *Artemia* originating from MIC-F-conditioned environments were supplied, *Bacillus* remained detectable until 56 days post-hatch.

These observations suggest that live food can function as more than a nutritional carrier or temporary delivery route. It can also contribute to conditioning the microbial environment of the larval tank.

> *"The true value of controlled live food is not only the microbiology it carries, but the microbiology it leaves behind."*
> **Tania De Wolf**

## Stability rather than immediate growth

The absence of significant differences in larval growth or quality should not be interpreted as an absence of biological relevance. Under well-controlled experimental conditions, larvae may perform similarly across different microbial management treatments. The potential value of a more stable microbial ecosystem may become more apparent under less favorable conditions, such as elevated organic loading, variable water quality, high densities, operational variation or increased microbial pressure.

The objective is therefore not necessarily faster growth under ideal conditions, but greater stability, resilience and predictability of the production environment.

## Towards controlled live food

Controlled live food combines robust production,

INVE AQUACULTURE

# FROM CLEAN LIVE FOOD TO CONTROLLED LIVE FOOD

## CONTROL MICROBIAL DEVELOPMENT



Sanocare™ SURE

SEP-Art®

D-FENSE®

## CONDITION THE MICROBIAL ENVIRONMENT




Sanolife™ MIC-F

## STABLE AND PREDICTABLE



## 4 PRINCIPLES

- CONTROL THE MICROBIAL STARTING POINT
- MAINTAIN MICROBIAL CONTROL THROUGHOUT LIVE FOOD PRODUCTION
- CONDITION THE MICROBIAL ENVIRONMENT
- EXTEND MICROBIAL MANAGEMENT INTO THE LARVAL ENVIRONMENT

## MICROBIAL MANAGEMENT: KEY FINDINGS

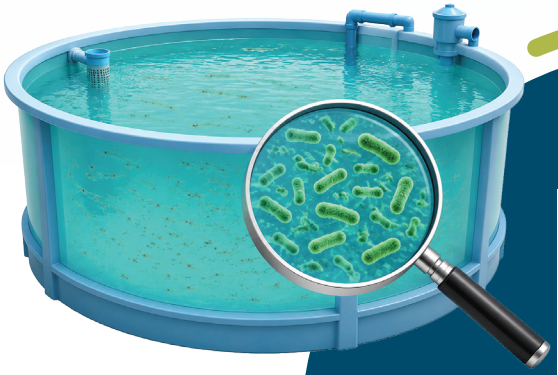
### BENEFICIAL BACILLUS BECOME PART OF THE MICROBIOME

*Bacillus* associated with rotifers and *Artemia*

### CONDITIONING OF THE MICROBIAL ENVIRONMENT IN THE TANKS

Persistent *Bacillus* detection in tank biofilms





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NUTRITION HEALTH ENVIRONMENT



nutritional optimization and deliberate microbial management.

SEP-*Art*® technology supports the production of physiologically intact *Artemia* suited to handling, enrichment, harvesting and storage. D-FENSE technology and Sanocare™ SURE help control undesirable microbial development. Sanolife™ MIC-F conditions the live food production environment with selected beneficial *Bacillus*, which can subsequently become associated with rotifers and EG *Artemia*. In this way, live food provides a biological connection between the conditioned live food environment and the microbial community of the larval rearing system.

These technologies are not the strategy in themselves. They are tools within a broader objective: making live food quality, and particularly microbial quality, less stochastic and more deliberate.

Hygiene, exclusion and disinfection will remain fundamental to hatchery biosecurity. But increasingly, they can be complemented by selective microbial management.

The result is more than cleaner live food. It is live food with a deliberately managed physical, nutritional and microbial profile.

That is the transition from clean live food to controlled live food, and towards a more stable and predictable larval rearing environment.

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