

## Boosting Hatchery Success: Effective Live Feed and Microdiet Protocols for Larval Rearing Efficiency

In marine fish larval rearing, the early stages are crucial for determining juvenile health, quality, robustness and performance during on-growing. This initial period is influenced by an extremely complex interplay of factors, that, among others, includes egg quality, environmental conditions and of course, feeding and nutrition.

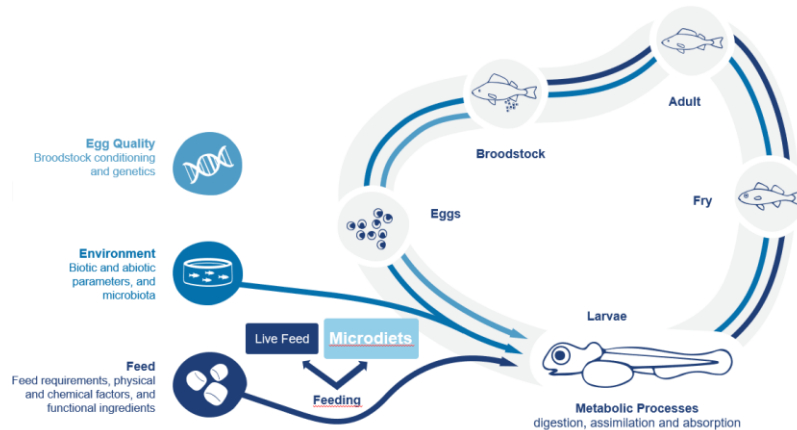


Figure 1. Main factors influencing fish larval development

A holistic farming approach, that considers and integrates all these elements is essential for hatchery success. This involves providing well-balanced nutrients, optimizing feeding strategies, and maintaining the best possible rearing conditions. Only by addressing these, can hatcheries significantly improve their operation and produce the best possible juveniles. This article focuses on proven feed and feeding strategies to help hatcheries achieve better results and reduce production costs.

### For Starters, Focus on live feeds

Rotifers and Artemia have been the predominant live feeds in the aquaculture industry since the 1970s. Their consistent use has facilitated the development of the industry, ensuring a steady supply of fry to meet increasing demands. However, it is important to note that both rotifers and Artemia inherently lack sufficient levels of Omega-3 highly unsaturated fatty acids (HUFAs), specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These organisms do not have the capacity to synthesize these fatty acids de novo, thus there is a need for enrichment to enhance their nutritional profile and meet the requirements of the newly hatched larvae.

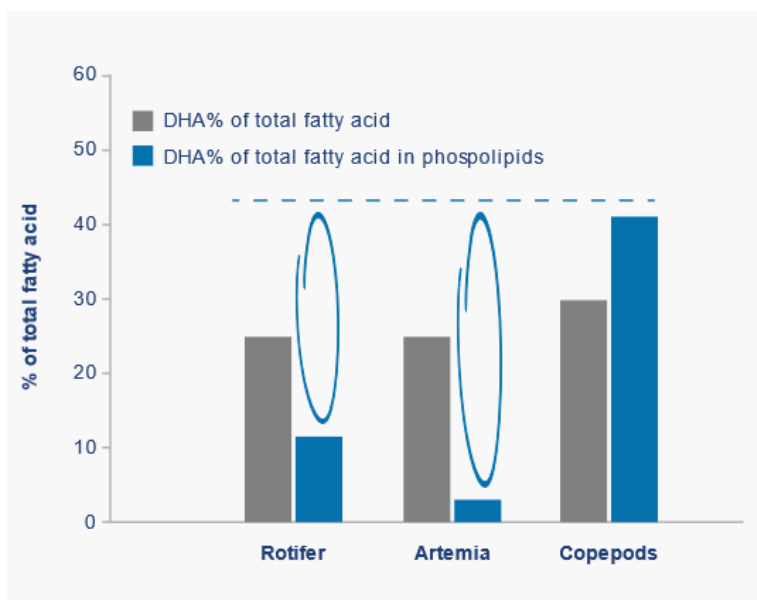


Figure 2. DHA% in the total lipids fraction (grey) and in the phospholipid fraction (blue) of enriched rotifer and Artemia compared to natural copepods

Several studies underscore the importance of incorporating DHA and EPA into the phospholipid fraction of the feed, as this form is significantly more bioavailable for larval growth and development compared to neutral lipids (triacylglycerols). High DHA and EPA in the phospholipid fraction is naturally present in the copepods that many fish larvae prey upon. Rotifers and Artemia contain similar amounts of phospholipids as copepods, but the proportion of those phospholipids represented by DHA and EPA is much higher in copepods than in rotifers and Artemia. While it is possible to elevate DHA levels in total lipids through enrichment with many of the available commercial products, the phospholipid content often remains inadequate for the nutritional needs of developing larvae.

LARVIVA Multigain, BioMar’s complete live feed enrichment formula, has been designed and optimized over the years to enrich rotifers and Artemia with all the essential nutrients required by marine fish larvae. It contains optimal levels and ratios of Omega-3 and Omega-6 fatty acids to ensure a balanced nutrition, enhanced with adequate levels of vitamins, key minerals, immunostimulants, and phospholipids, to support robust health and growth. Additionally, it features Bactocell®, the only probiotic authorized for use in aquaculture feeds within the EU, promoting a healthy gut microbiome with documented effect on deformity reduction.

### Live Feed Enrichment Trials

In order to extract the full potential from LARVIVA Multigain, BioMar has performed a series of trials with both rotifers and Artemia to develop live feed enrichment protocols that optimize the levels of Omega-3-HUFAs in both total lipids and - more importantly - on phospholipids, ensuring the best possible nutrition for fish larvae.

In the trials, the effects of different variables (temperature, enrichment dose, enrichment duration and live prey density), on fatty acid enrichment in rotifers and Artemia were assessed.

## Enrichment trial Results

Both in rotifers and in Artemia, the results indicated that the duration of enrichment and the dose of LARVIVA Multigain used were critical for optimal fatty acid incorporation into phospholipids.

Based on the findings, when using LARVIVA Multigain, a long (18-20h) enrichment protocol is recommended to allow sufficient time for DHA to incorporate into phospholipids, with higher doses leading to greater enrichment effects, while density and temperature can be adjusted as needed. Once enriched, storing the live prey at 4-8°C for up to 24h has proven to be a viable option with no major impact on live-prey nutritional value.

Additionally, making practical use of the extensive data collected, BioMar has developed a computer model that allows hatcheries using LARVIVA Multigain to adjust parameters to predict enrichment outcomes or set desired phospholipid DHA levels and receive recommendations for optimizing the enrichment process to meet specific needs and environmental conditions.

## Moving onto Microdiets

As the perfect complement for optimally enriched live prey, the transition to a high quality microdiet will have a profound effect on the outcome. But as it happens with the enrichment, for the best potential results in any hatchery, using the best microdiet in the market will not be enough. Feed and feeding need to be well adapted to the technical characteristics of the facility, as well as to the physical behavior of the feed that is being used.

LARVIVA ProStart is BioMar's premium larval feed, with a manufacturing process that ensures nutrient preservation and maximizes physical quality. It is designed to support early larval development up to the post weaning phase, it is nutritionally formulated and balanced to comprehensively fulfil all nutritional requirements of marine fish larvae and, as LARVIVA Multigain, it includes the probiotic Bactocell with a proven positive effect on nutrient assimilation in fish.

For the best potential results, as was done for the enrichment, BioMar conducted a large scale trial with seabream larvae to optimize LARVIVA ProStart feeding protocols. The trial tested various feeding strategies with LARVIVA ProStart and standard diets to determine their effectiveness in promoting larval growth and health.

Four different feeding strategies were evaluated:

1. **Treatment 1 (Early Intro-Prolonged ProStart):** LARVIVA ProStart was introduced at first feeding (3 DPH, Days-Post-Hatching) and continued all the way down to Post Weaning (45 DPH), ensuring the availability of the best possible diet from mouth opening until the pre-juvenile phase.
2. **Treatment 2 (LF Intro-Prolonged ProStart):** LARVIVA ProStart was introduced right after swimbladder inflation (15 DPH) and continued all the way down to Post Weaning (45 DPH), relying on live-feeds for the initial stages (3DPH-15DPH) and ensuring the availability of the best possible diet until the pre-juvenile phase.
3. **Treatment 3 (Standard):** LARVIVA ProStart was introduced after swimbladder inflation (15 DPH) and continued down to 31 DPH, relying on live-feeds for the initial stages (3DPH-15DPH), providing a strong microdiet for the critical weaning phase (16DPH-30DPH) and transitioning to a standard and less costly diet for the post-weaning phase (31DPH-45DPH).



- 4. **Treatment 4 (Std Double LF):** Like Treatment 3, but with double the amount of Artemia, under the assumption that more Artemia could serve as a complement to the use of a lower quality diet during the critical weaning phase.

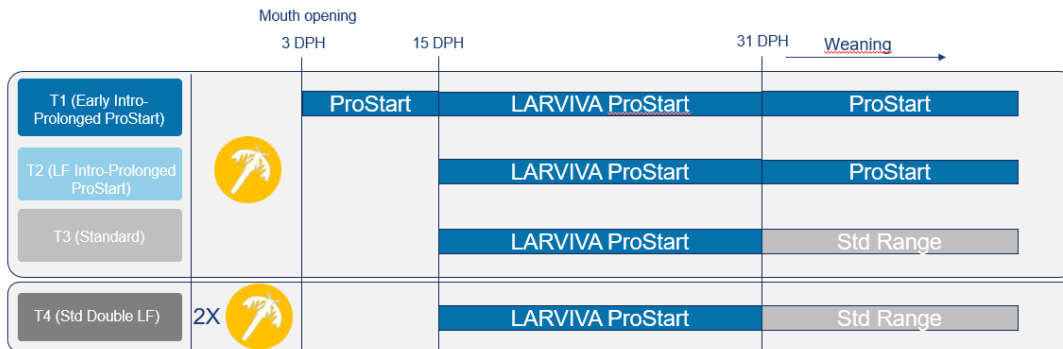


Figure 3. Diagram of the different treatments included in the LARVIVA ProStart trial

The trials were conducted on seabream larvae, from 3 to 45 dph at the larval facilities of Ecimat (Estación de Ciencias Mariñas de Toralla, Vigo, Spain), using standard industrial densities and conditions at pilot-scale 400-liter triplicate tanks.

Throughout the trial, the parameters used to evaluate the performance of the different groups were survival rate, deformity rate, growth, and the evolution of digestive enzyme activity, which is a crucial indicator of the digestive system’s development in fish larvae. Higher enzyme activity suggests better digestion and nutrient absorption, which are essential for growth and overall health.

With all the data generated in the trial, and with the costs of the different feeds and live feeds, a direct-cost-efficiency analysis was conducted to identify the most effective feeding strategy.

### Microdiet trial Results

No significant differences in survival rate were observed among the different groups.

In terms of deformity, the groups with an extended use of LARVIVA ProStart (Treatments 1 & 2) showed significantly lower deformity levels than the groups that move early to a standard diet (Treatments 3 & 4).

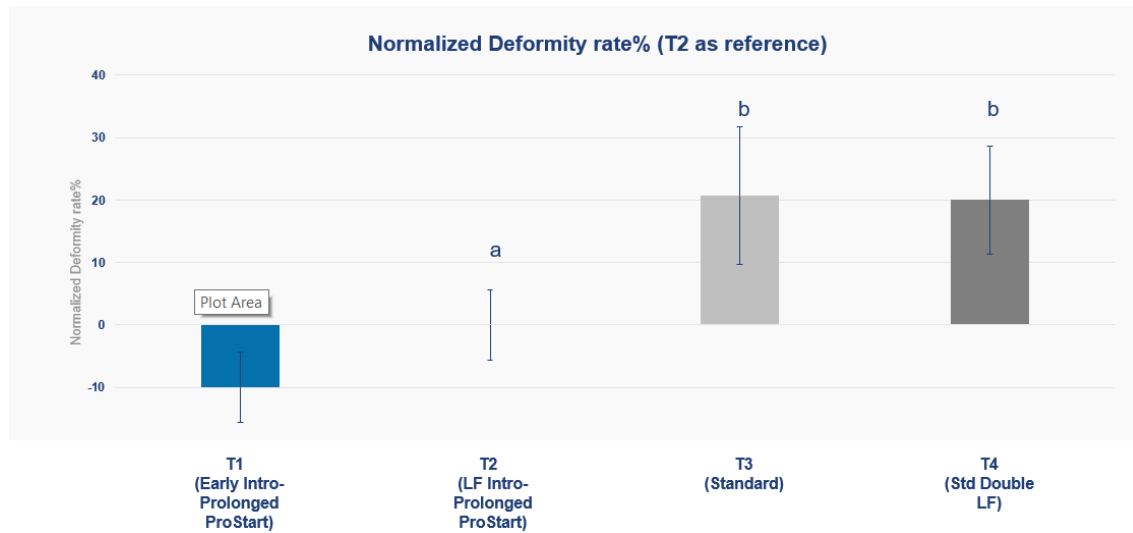
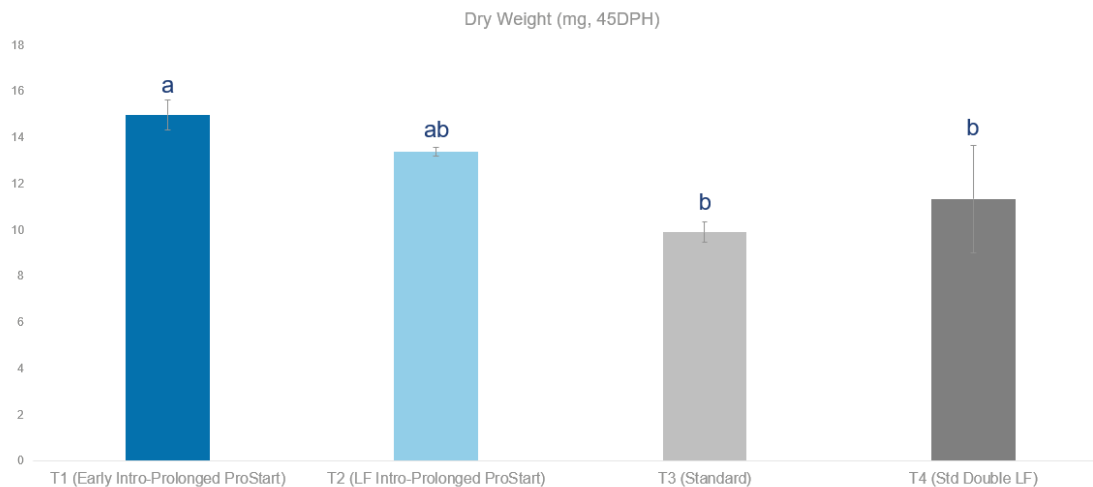


Figure 4. Normalized deformity rate, using “*LF Intro-Prolonged ProStart*” (Treatment 2) as a reference.

Similarly, for growth, data indicates that larvae fed with Larviva ProStart through weaning (Treatments 1 & 2) had significantly higher dry weights at 45 dph compared to those fed the standard range (Treatments 3 & 4) .



Larval average dry weight at 45 DPH.

Figure 5.

When monitoring the activity of digestive enzymes (phosphatase, aminopeptidase, trypsin, chymotrypsin and lipase) as indicators of digestive system maturity.

- Larvae fed with LARVIVA ProStart from 3 dph (Treatment 1) exhibited significantly higher aminopeptidase and Chymotrypsin activity compared to those fed the standard range (Treatment 3), suggesting a higher ability to break down proteins into amino acids, which are vital for growth and development.
- Additionally, a higher activity of Trypsin, amylase and lipase was observed in the larvae fed with LARVIVA ProStart from 3 dph (Treatment 1), suggesting a higher ability of this group to utilize carbohydrates from the diet and to break down fat into fatty acids like DHA and EPA.



The higher digestive enzyme activity in larvae fed with LARVIVA ProStart from 3 dph suggests a more advanced and efficient digestive system. This likely contributed to the improved growth performance and lower deformity rates observed in this group.

**Results summary**

The findings from this trial unequivocally indicate that the prolonged use of LARVIVA ProStart until the end of weaning enhances larval performance over the use of standard diets at the late weaning phase. Furthermore, indications from this trial and previous experience point towards the early introduction of microdiet as a beneficial strategy to accelerate digestive system maturation translating into improved performance.

**Direct-Cost-Efficiency-Analysis**

Just as in any other economic activity, achieving cost efficiency is key in the production of marine fish larvae and juveniles. In order to address this aspect, a cost-determination exercise was done for each treatment. The cost of feeding consumables (artemia cysts, enrichment products, dry feeds) plus the costs of hiring extra staff for removing excess deformities, were combined with the final amount of good quality larvae to obtain a direct-cost analysis.

The results (figure 5), indicate that extending the use of LARVIVA ProStart throughout the entire larval and weaning cycle results in very significant reductions in costs. Despite being more expensive than standard diets, adopting prolonged LARVIVA ProStart strategies remain cheaper overall than strategies which rely on standard diets for weaning.

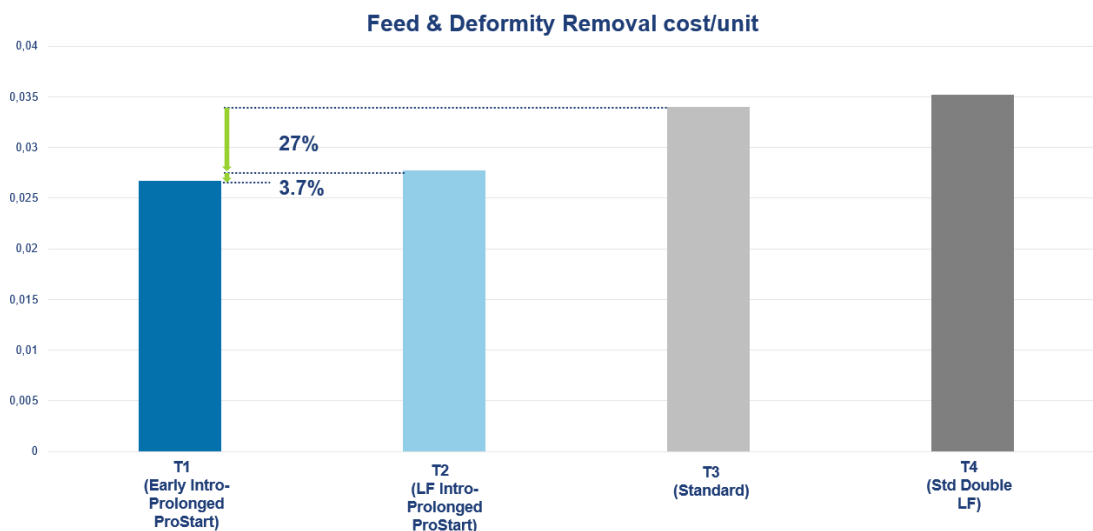


Figure 6. Direct-Cost-Efficiency-Analysis results



## Conclusion

Producing good quality marine larvae is among the greatest challenges that can be found in animal rearing. The complex interplay of factors affecting culture units forces producers to pay enormous attention to detail and to control every aspect of hatchery operation.

Feed and feeding are paramount to achieve this success, starting by the enrichment of rotifers and Artemia with the required fatty acids, particularly in phospholipids, which is crucial for meeting the nutritional needs of fish larvae. LARVIVA Multigain has been designed with this in mind, and the results here presented have served as the base to develop the optimal enrichment protocols for each hatchery to achieve optimal fatty acid levels in their live feed.

Along with the live feed, the introduction, transition and weaning onto dry diets will be instrumental drivers of juvenile quality, the use of LARVIVA ProStart, a top quality microdiet, during larval rearing and weaning has proven to not only enhance culture performance, but to do it while significantly reducing production cost when compared to weaning with feeds at a reduced price. Additionally the early introduction of LARVIVA ProStart at mouth opening, contributes to further improving results by promoting an early maturation of the digestive system.

In conclusion, the use of LARVIVA Multigain and LARVIVA ProStart, and the adoption of effective live feed and microdiet feeding protocols are powerful tools for boosting hatchery success. By providing well-balanced nutrients, optimizing feeding strategies, and maintaining the best possible hatchery conditions, hatcheries can significantly improve the survival rates and overall health and robustness of fish larvae. A strong start is indeed the key to sustained success in aquaculture.

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LARVIVA, Start strong. Stay strong.