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Latest advances in feeding early life stage and broodstock aquatic species

Rotifer culture diet eliminates need for enrichment

Nutritionally optimized rotifers for larval feed

Copepods

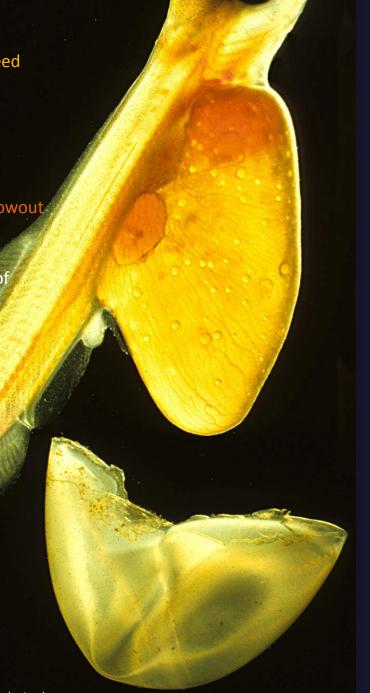
Bridging the gap between hatchery and growout

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Breakthrough rotifer culture diet eliminates the need for enrichment

Eamonn O'Brien, Product Manager for Skretting's Marine Hatchery Feeds (MHF) reveals the company's new rotifer culture diet, which is about to be launched on the market.

For the last quarter of a century, hatcheries have tended to follow the same labour intensive batch culture system of rotifer production. This process, which usually observes three - or four-day batches, follows a lengthy sequence of events, whereby rotifers are harvested, then rinsed, then put into an enrichment tank for any number of hours, after which they are harvested again, then cleaned and finally fed to larval fish.

However, the more rotifers are handled, the greater the risk becomes of damaging them. Furthermore, the fish are normally fed rotifers three to four times a day, which often requires the same number of separate rotifer harvesting and enrichment processes.

In order to verify the hypothesis that a well formulated live food diet could avoid the use

of an enrichment step, Skretting's Marine Hatchery Feeds (MHF) conducted extensive trials in which it transformed its existing rotifer culture diet ORI-CULTURE to slowly release the enrichment components, such as protein, fat, vitamins and minerals in non-suffocating concentrations.

The results were conclusive. Skretting found that by naturally-enhancing rotifers during the culture period, they could be taken at any stage during the culture and fed directly to larval fish. Not only does this enable hatcheries to establish more efficient production systems, but because the rotifers will not be subjected to



Rotifer fed on ORI-ONE

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an enrichment phase and are handled less, they are inherently cleaner and healthier.

These findings have led to ORI-ONE, a new algae-based powder for rotifer production, which is being launched at the Aquaculture Europe conference, held in Trondheim, Norway, from 9-12 August, 2013.

"ORI-ONE could revolutionize the entire production process within the rotifer room, streamlining production and increasing efficiency while further improving rotifer quality, which is essential for successful larval rearing production," said Eamonn O'Brien, Product Manager for Skretting's Marine Hatchery Feeds (MHF).

Skretting also found ORI-ONE could outperform its own market leading conventional diet and enrichment process, comprising ORI-CULTURE and ORI-GREEN. With a slow enhancement over the culture period, ORI-ONE naturally enriched rotifers were to the same level as following the conventional regime. After one cycle of three-to-four days, the enrichment seen was 100 percent that of the conventional procedure.

The experiment was conducted over several culture cycles to confirm the diet did not compromise the health or reproduction of the rotifers.

With hatcheries confronted by soaring production costs, ORI-ONE could not be launched at a better time.

"Energy and oxygen costs in particular are soaring and rotifers are enriched at 26 degrees Celsius, which requires hatcheries to heat a lot of water for both the enrichment and sub-



ADVANCED CULTURE & ENRICHMENT PRODUCT FOR ROTIFERS





sequent rinsing steps. ORI-ONE significantly reduces that requirement and also simplifies the processes for hatchery managers so they can focus more on the quality aspects of cultivating rotifers," said O'Brien.

ORI-ONE also results in significantly more stable rotifers. Following the conventional enrichment process, rotifers are put into a very eutrophic environment filled with emulsions or fats, which are detrimental for the rotifer. But with ORI-ONE, hatcheries are feeding the rotifers an algae feed which they incorporate into their own biomass.

Nutrients being present at metabolizing concentrations allowed not only gut tissue filling but also gradually enriched the tissue of the rotifers without consuming excessive oxygen or eutrophication, which is a well-known problem under conventional enrichment conditions. Following the ORI-ONE regime, the nutrients are naturally incorporated into the rotifer tissue, making their dietary value much more stable.

In addition, removing the enrichment step all but eliminates the emulsion discharges that can be released into the environment during post-enrichment rinsing.

ORI-ONE follows the same principles as the rest of Skretting's ORI-GO range of rotifer diets and enrichments. This includes a wider particle size range, which results in a much more efficient uptake. The particles are also in a wide variety of shapes, which gives better adhesion and retention within the rotifers.

"We introduced a lot of new ideas to the market when we launched ORI-CULTURE six years ago; these concepts have since been universally adopted and the product is market leader. ORI-ONE is another milestone product – all the benefits of ORI-CULTURE are incorporated into the product so it fits comfortably into the ORI-GO product portfolio, but it also represents the natural progression of the rotifer culture diet," said O' Brien".

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For more information, please contact <u>Eamonn O'Brien</u>, Product Manager, Skretting Marine Hatchery Feeds (MHF)

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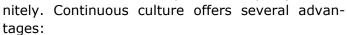
Fundamental Principles for Production of Nutritionally Optimized Rotifers for Larval Feed

Eric C. Henry, Ph.D., Research Scientist, Reed Mariculture Inc. discusses rotifer enrichment strategies.

Rotifers are established as the preferred feed for larvae of many aquacultured fish and crustaceans, because rotifers are the only live zooplankton that can be reliably cultured in mass quantities. Numerous studies have assessed various methodologies for producing rotifers and enhancing their nutritional content, so it is possible to outline some fundamental principles that are key for effective and economical rotifer production:

Continuous vs. batch culture — Stability promotes rotifer health and consistent productivity

Many hatcheries produce rotifers in "batch" cultures, which are grown to a maximum target density and harvested completely. The culture tank is then cleaned, sanitized, and a new culture is started. With "continuous" cultures, once the culture reaches the target density only a fraction (typically 20–50%) is harvested, and this harvest can be repeated every day, indefi-





- Once harvesting begins, the culture requires the same feeding and harvest every day, simplifying the management of culture operations and so minimizing the opportunities for costly mistakes.
- Rotifers are most productive under stable conditions. The consistent feeding and harvest regimes that prevail in continuous culture promote rotifer health, supporting high productivity and nutritional quality.
- The rotifers have a younger age distribution, due to the high daily harvest rate. Younger rotifers feed more actively, are more fecund, and are more vigorous swimmers.
- There is no interruption of production while a new culture grows to harvest density.

Labor inputs are reduced because culture tanks do not require frequent sanitizing and re-inoculation.

Intensive vs. extensive culture — Small is beautiful

Intensive cultures (3 –10 million rotifers per liter) require smaller culture volumes and so less space in hatcheries. They are readily enclosed, allowing control of culture conditions and exclusion of contaminants. They require less water, and more concentrated rotifers are easier to harvest. High-density cultures require high-quality, high-density feeds (concentrates). The consistent concentration of these feeds enables automated delivery to the culture by metering pump.

Grow-out enrichment vs. supplemental enrichment — Too much, too late

The nutritional value of rotifers depends on what they are fed. Typically, a batch culture is grown to harvest density using a low-cost yeast-based feed of low nutritional value, and then switched to a high lipid content "enrichment" feed a few hours before harvesting. This "gut loading" strategy fills the digestive tracts of the rotifers with the lipid-rich feed, to be delivered to the larvae when the rotifers are consumed. However, the extreme lipid

A more effective enrichment strategy is to grow the rotifer culture on a more moderate rotifers are not stressed, so they show good motility

content of conventional enrichment feeds is stressful to the rotifers, harming their health and motility. Lipid emulsions foul rotifers, requiring them to undergo a washing procedure before feeding to larvae. Harvesting on enrichment feed, so that the entire screens, washing procedures, and temperature body of the rotifer is enriched. The shocks when enriched rotifers are "cold banked" before feeding to larvae can cause the rotifers to eject their gut contents (and enrichment) before they are fed to larvae.

A more effective enrichment strategy is to grow the rotifer culture on a more moderate enrichment feed, so that the entire body of the rotifer is enriched. The rotifers are not stressed, so they show good motility. They require no washing, and can be fed directly into the larval tank, eliminating the labor of harvesting and washing, and avoiding shocks that cause ejection of the gut contents. Rotifers enriched during grow-out retain their enrichment in the larval tank and can deliver more enrichment to the larvae. If the levels of "grow-out" enrichment are below desired levels, a less extreme (less stressful, less fouling) supplemental enrichment is then sufficient.

Liquid vs. dry feeds — Aquatic feeds for aquatic organisms

Dry feeds are attractive because they are less costly to ship and easier to store than liquids, but they have several disadvantages. They can be more difficult to feed by automation; they must be thoroughly dispersed in water before use, often by strong mechanical mixing; feed particle size (critical for rotifers) can be compromised by clumping, which can also foul the rotifer tank; water-soluble components begin to leach from particles as

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soon as they are hydrated. Liquid feeds usually require no preparation and can be pumped directly into the rotifer tank; feed particles stay well-dispersed; the feed can incorporate microalgal cells not distorted and denatured by drying.

Algae-based vs. emulsion-based enrichments — The advantages of Natural Foods

Oil emulsions are commonly used in enrichment feeds to boost the HUFA (Highly Unsaturated Fatty Acid) content of rotifers. Oil emulsions tend to foul rotifer cultures, and can be biased toward triglycerides, good energy sources but lacking the membrane phospholipids needed for larval growth, and an over-emphasis on lipid enrichment has caused the protein content of some feeds to be neglected. Feeds based on microalgae, the natural food of rotifers, provide a balance between lipid (with a high phospholipid content) and protein, naturally microencapsulated in appropriate-sized particles—the cells of selected microalgae.

Reed Mariculture has developed algae-based RotiGrow® enrichment feeds for both rotifer grow-out and supplemental enrichment. Intact whole cells ensure that rotifers receive the maximum nutritional benefit from our exceptional feeds, which in turn provides maximum nourishment to your larvae. Our feeds also produce clean rotifers that ordinarily do not require washing, so they can be fed directly into the larval tank.

RotiGrow Plus® is the grow-out enrichment feed that provides all these advantages:

- Healthy Rotifer Cultures Rapid growth, minimal fouling and reduced bacteria loads
- Higher Yields Rotifers can double daily at densities over 5,000 L-Type rotifers per mL
- Essential Omega Boost Provides 50–100% of the Omega-3 fatty acids needed by the larvae of most marine species — without secondary enrichment!
- Optimum Enrichment Supports full highlyunsaturated fatty acid (HUFA) enrichment, full vitamin enrichment with proper protocols, and additional carotenoid enrichment of rotifers without sacrificing



other nutritional factors or stressing the rotifers

 Concentrated, Clean, Liquid Form — Exceptionally clean and easy to use with minimal foaming or clumping, which means less time spent maintaining cultures

RotiGrow N-Rich® feeds provide still higher enrichment using short-term protocols that produce clean, healthy and vigorous rotifers. This chart shows the composition of rotifers enriched using various RotiGrow enrichment protocols:

ROTIFER COMPOSITION

Essential Fatty Acids and Total Lipids Typical profiles of rotifers grown on RotiGrow *Plus* and enriched using N-Rich *High PRO*, a protein-rich feed, and N-Rich *PL Plus*, a phospholipid-rich feed.

_	_					
	RotiGrow Plus	2-hour enrichment N-Rich <i>High PRO</i>	8-hour enrichment N-Rich <i>High PRO</i>	2-hour enrichment N-Rich <i>PL Plus</i>	Cod eggs	Cobia eggs
Protein	68%	66%	64%	65%	79%	n/a
DHA	17	30	40	36	33	42
EPA	12	13	12	10	18.6	13.5
ARA	1.9	2.0	2.0	1.9	2.2	3.4
HUFAs	43	52	58	51	56.2	68
Total Lipids	10.5%	12.5%	14.2 %	12.6%	13.2%	22%
Polar lipids	54.0%	55	55.7%	55	71%	n/a
Sterols	4%	4.2	4.3%	4.0	9.8	n/a
Free fatty acids	5.9%	5.1	4.9%	5.0	5.4	n/a

NOTE: fatty acid units after "profile" units are mg/g d.w.

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ABOUT THE AUTHOR

Dr. Eric Henry earned his Ph.D. in phycology (algae studies) at the University of British Columbia and continued academic research on algae for the next 16 years, authoring or co-authoring 25 research papers in scientific journals. In 1996 he joined the private sector to develop algal mass culture and associated aquaculture technologies, and he has been part of the Reed Mariculture team since 2003. Eric works on algal and zooplankton culture, development of new products, and provides customer technical support relating to uses of Instant Algae, rotifer culture, and larviculture of shellfish and finfish.

For more information, contact Eric C. Henry, Ph.D.

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COPEPODS

Nature's Choice



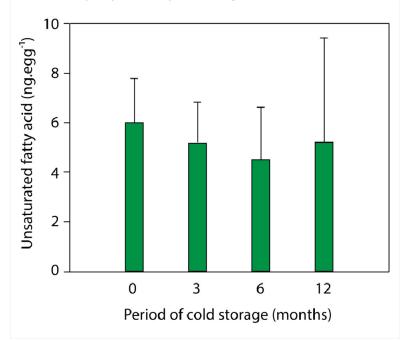
Professor Benni Winding Hansen and co-workers describe work with copepods as live feed for fish larvae. The research effort focuses on a unique egg storage technique, biochemical quality of copepod eggs and the hatched live feed nauplii larvae on a boreal copepod species as well as perspectives on a tropical copepod species currently being investigated.

Copepods are probably the most numerous animals on earth, with more than 12,000 known species. Of prime importance in many marine ecosystems are copepods from the order Calanoida. Many calanoid copepods are herbivorous and feed on phytoplankton, and are

preyed upon by numerous fish species and are often the first feed for many marine fish larvae. Hence: 'copepods; natures choice'. An interesting trait regarding calanoid copepods is that the eggs can be provoked into a resting stage, where the egg can be stored, similar to brine shrimp (Artemia) cysts. This method is the most promising storage technique for distribution of copepod eggs to aquaculture facilitates world wide. The eggs can be hatched and the nauplii larvae can be fed to marine fish larvae while still obtaining a high nutritional quality. The eggs can be stored for up to one year without a significant loss of unsaturated fatty acids (fig. 1).

The ability of copepod eggs to be

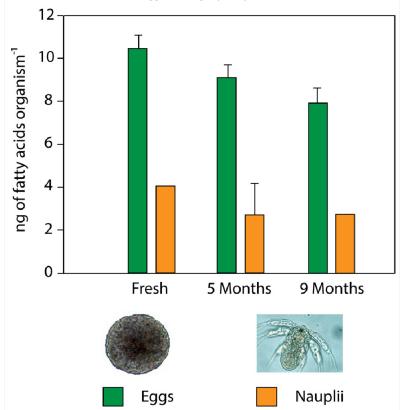
Figure 1 No significant loss in amount of fatty acid is observed during cold storage of copepods eggs. Validating that cold stored eggs do not lose nutritional quality after one year of storage.



stored is one aspect, but what about the nauplii being the ideal food for marine fish larvae? When eggs are compared to live nauplii the eggs contain a significantly higher amount of fatty acids than the hatched nauplii. This is a result of the natural process of fatty acids being used during the hatching process, as an energy source. The hatched nauplii do not exhibit a significant difference in fatty acid content over time though, suggesting that nauplii from cold stored eggs are equally as good as live feed as nauplii hatched from fresh eggs. This validates that copepod eggs can be stored for at least 9 months and the nauplii still can be used as high quality feed for aquaculture marine hatcheries (fig. 2).

It is common knowledge that copepods are better food

Figure 2 No significant loss of fatty acids were observed between nauplii hatched from fresh eggs or from cold stored eggs. Validating nauplii hatched from cold stored eggs as a high quality feed item.



items than traditional live feed, which are rotifers (*Brachionus* spp.) and *Artemia* spp. Copepods are almost always a better nutritional choice than even enriched rotifers and *Artemia*. In terms of fatty acids, to ensure an adequate nutritional food package for fish larvae, levels and ratios of the essential fatty acids Eicosapentaenoic Acids (EPA) and Docosahexaenoic Acids (DHA) are important. The proportion of DHA and EPA are better in copepods than in rotifers (Fig. 3), but can differ significantly from one copepod to another. This allows different copepods to be used as feed for fish larvae from different fish species.

The ability to provide fish larvae with an adequate fatty acid content through live feed is, even by using copepods, not trivial because the fatty acid composition in copepods depends on various factors. The most influential factor is the transfer of fatty acids from algae to the feeding copepods, which typically creates a fatty acid profile that is representative of the profile found in the food particles. Even copepods of the same species can exhibit profound differences in fatty acid profiles due to the differences in food particle regimes found in the particular systems of which the copepods are located. It is therefore of no surprise that optimizing the nutritional value in live feed can be achieved by providing the copepods with food particles containing a desirable fatty acid content. However, there are certain additional factors that govern copepods fatty acid profiles that must be considered. Those that are of particular interest are the copepods food particle size selec-

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tivity and their possible fatty acid chain alteration capabilities.

ΑII species of copepods undergo different stages of development and thereby have different feeding characteristics. Many copepod species do not feed on food particles until reaching advanced development stages, meaning that they solely rely on the lipids content allocated to them from the female adult throughout their juvenile stages of development. This can result in a fatty acid profile that is distinctly different from adult to nauplii depending on which fatty acids are allocated and which are metabolized. Even if the juveniles are able to ingest food particles, which

Figure 3 Different proportions of the essential fatty acids EPA and DHA in two different copepods and rotifers. As seen on the figure copepods are superior with larger amounts of EPA and DHA, when compared to rotifers.

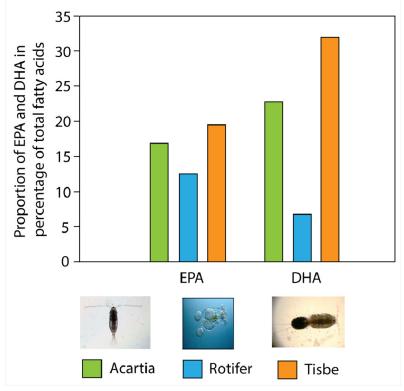
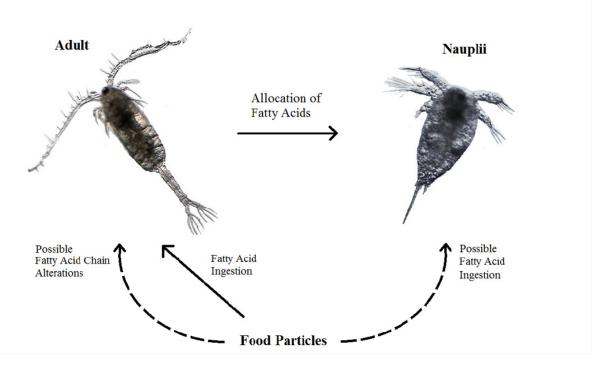


Figure 4: Illustrating the dynamics of fatty acid transfer from the copepods food source to the adult and nauplii.

The copepods that are illustrated are pictures taken of the tropical calanoid copepod *Pseudodiaptomus annandalei*.



is the case for most copepods e.g. under the genus *Pseudodiaptomus*, their size, in comparison to the food particles, could prohibit them from ingesting the same diet at the adults. Coupled with the possibility of providing existing fatty acids with additional hydrocarbons and double bonds, a phenomenon known as chain elongation and desaturation, the fatty acid profile between the food source, adults and nauplii can have distinct differences (figure 4). Elongation and desaturation have been observed in adults from different species and could have the potential of enhancing the existing fatty acid content from its food. Thereby, the discussion on optimizing the nutrition value by providing live feed with algae with optimal fatty acid contents does not stop here. Considering a copepod candidate and its food source for the production of live feed should also be done on the basis of predator-prey interactions throughout its development and its biochemical enhancement capabilities. In doing so, producers of live feed will be able to select species of copepods and algae that are best suited as feed for a particular fish species of commercial interest; one could call it 'designer feed' for finfish larvae.

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ABOUT THE AUTHORS

Professor Benni Winding Hansen has been working with copepods for more than 25 years and is the coordinator of 'IMProvement of AQuaculture high quality fish fry production' (IMPAQ) and partner in 'COpepod egg Mass production in Aquaculture' (COMA). The research in live feed is based at the Department of Environmental, Social and Spatial Change (ENSPAC) at Roskilde University, Denmark, and hosts the research initiative 'Biological Production'. Besides working on semi-intensive outdoor copepod production, Biological Production work with optimization of phytoplankton production in bioreactors for use in recirculating copepod production.





The team members are from left to right: Benni Winding Hansen, Guillaume Drillet, Per Meyer Jepsen, Thomas Allan Rayner.

This work focuses on biological and technical solutions for mass cultivation of copepod eggs for live feed production in the aquaculture sector. The project works in close collaboration with DenSelect, hosted in DHI Group Water and Environment, Singapore by Business Development Coordinator for Aquaculture by Dr. Guillaume Drillet.

The copepod work is financed by SFR the Danish Strategic Research Council and by the High Technology Foundation (contact) and DHI are financed by the Danish ministry for independent research (contact).

For more information contact <u>Professor Benni Winding Hansen</u>, Roskilde University, Denmark – Department ENSPAC.

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OPTIFeed Technology

A novel in vitro system for accurate prediction of biological larval feed performance

OPTIFeed Technology can help move the aquafeed industry from formulating for Feed Conversion Ratio to Economic Feed Conversion Ratio, according to Katerina A. Moutou, Department of Biochemistry & Biotechnology, University of Thessaly, Greece.

Larval feeding

The capacity to produce robust juveniles has been pivotal for the rapid industrialization of the aquaculture sector worldwide and still is a critical factor towards increased competitiveness and sustainability. Despite major achievements in the field, the formulation of larval feeds that deliver timely sufficient nutrient quantities to support the rapid development and growth of fish larvae, remains a quest that requires intensive endeavors and big investments. The introduction of new fish species, the volatile market of raw materials and the demand for the earliest possible weaning define a highly challenging environment in the field of larval nutrition, where nutritional requirements are largely unspecified and tools to determine nutritional quality of larval feeds are lacking.

OPTIFeed Technology

OPTIFeed Technology optimized the pH-stat methodology for protein digestibility *in vitro* to determine the nutritional value of larval formulations for different developmental stages and marine species. Varied quantities of feed and digestive enzymes react in an environment of controlled temperature, salinity and pH in order to simulate the conditions prevailing in the digestive tract taking into account differential consumption rates. OPTIFeed Technology utilizes crude enzyme extracts isolated from different fish species and larval stages to compare, predict and benchmark the performance of raw materials and larval formulations. OPTIFeed Technology revolutionizes our approach to larval nutrition providing cost- and time- effective measure of biological performance otherwise impossible to obtain:

√ Rate of dietary protein autohydrolysis

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- √ Effect of temperature on digestibility
- √ Effect of feed consumption on digestibility
- \checkmark Evolution of digestive capacity as the digestive tract and glands mature during larval development
- ✓ Effect of feeding regime on shaping digestive capacity

The potential of OPTIFEED Technology can assist larval fish feed companies to:

- Optimize the selection of raw material based on biological performance and cost
- Benchmark of prototype feeds versus the competition
- Evaluate the performance under variable conditions before actual feeding the fish
- Achieve faster product development based on reliable in vitro testing
- Customize feeds on nutritional needs (species, stage, period) and
- Reduce customer complaints about performance

Prediction of growth performance: coupling OPTIFeed Technology and *in vivo* trials

It recently became evident that OPTIFeed Technology can provide reliable comparative estimates of the growth performance of fish larvae fed on different diets. Two trials run in collaboration by AQUARK, Bernaqua – INVIVO NSA and Dr Katerina Moutou, University of Thessaly, Greece, for two different developmental stages of European sea bass showed the high predictive capacity of OPTIFeed analysis.

Trial 1: Feed formulations for early weaning

Two agglomerated feeds X vs Y with protein content 60% and 69% respectively were tested for early weaning in European sea bass larvae (9-45 days post hatch). OPTIFeed Technology determined *in vitro* a) the autohydrolysis rates of the feeds, b) the digestibility of dietary protein before and after stomach function was initiated. For that, crude enzyme extracts were isolated from European sea bass larvae of 10 and 29 dph and used to determine dietary protein digestibility. Feed X exhibited higher autohydrolysis rates per mg dietary protein compared with feed Y. Feed X exhibited also higher digestibility rates at 10 dph, whereas feed Y was more digestible by 29 dph (Figure 1). This interesting in-

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Figure 1. Autohydrolysis and *in vitro* digestibility of feeds X and Y tested for early weaning in European sea bass. Although feed X performed better than feed Y, its performance decreased with increasing age and maturity of digestive tract.

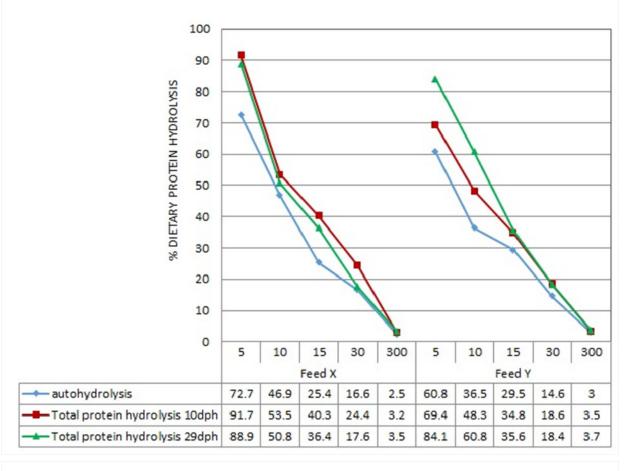


Table 1. Parameters determined in feeding trial 1 to compare feeds X and Y for early weaning in European sea bass

European sea bass Iarval feeds	Feed X	Feed Y	Feed X + <i>Artemia</i>	Feed Y + <i>Artemia</i>
Body weight 30 dph (mg)	3.0	2.5	9.4	8.8
Body weight 45 dph (mg)	35.5	30.1	100.6	99.2
Survival at 45 dph (%)	36.6	43.4	59.5	70.6
Total biomass at 45 dph (g)	31.2	31.4	143.7	168.1
Trypsin activity U/mg protein	0.049	0.028	0.076	0.054

version reflects the changes the digestive tract undergoes with maturation. According to OPTIFeed Technology Feed X was more appropriate for the early stages whereas feed Y properties were more suitable for the digestive apparatus after stomach function is initiated.

The results of *in vivo* feeding trial were in agreement with the findings *in vitro*; feeding of larvae on feed X up to 30 dph led to higher growth compared with feed Y, whereas feed Y

Table 2. Parameters determined in feeding trial 2 to compare four commercial post-weaning feeds in European sea bass

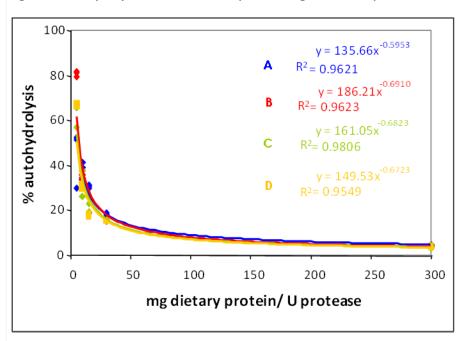
European sea bass	Тор	Second best	Third	Poor
larval diets	Performance	Performance	Performance	Performance
	Feed B	Feed C	Feed D	Feed A
Initial Weight 60 dph (g)	0.220	0.220	0.220	0.220
Final Weight 100 dph (g)	2.812	2.732	2.613	2.278
Specific growth rate (%)	6.89	6.81	6.69	6.32
Feed Conversion Ratio	0.70	0.71	0.72	0.77
Trypsin activity U/mg protein	0.0097	0.0095	0.0089	0.0076

fueled a higher biomass as a combination of body weight and survival, in the second period of the trial 30-45 dph (Table 1). The relative performance of two feeds did not differentiate when co-feeding with *Artemia* was performed from 9 dph to 30 dph (Table 1).

Trial 2: comparison of post weaning feed formulations

Four commercial formulations A, B, C and D with 56.7%, 63.1%, 64.4% and 54.4% protein content were compared for their performance in European sea bass of 60-120 dph. In this case, OPTIFeed Technology determined in vitro the autohydrolysis rates of the feeds and the digestibility of dietary protein with crude extracts enzyme isolated from European sea bass larvae of 80 dph. Feeds were rated

Figure 2A. Autohydrolysis of four commercial post weaning feeds in European sea bass.



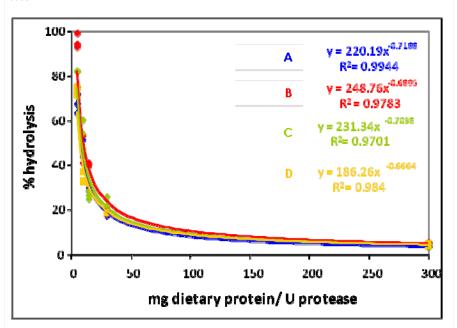
Autohydrolysis rates (%)							
mg dietary protein	5	10	15	30	300		
Feed B	80.8	35.9	23.5	15.5	4.1		
Feed C	63.2	31.3	22.8	15.5	3.5		
Feed D	66.8	30.4	17.7	15.0	3.6		
Feed A	44.7	38.0	30.5	18.5	4.3		

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according to OP-TIFeed Technology results; B was the top performer with the highest autohydrolysis and digestibility rates followed by C and D, whereas A exhibited the poorest performance (Figure 2).

Once more, OPTIFeed offered а reliable insight of the performance in vivo. European sea bass fed on feed B between 60 dph and 120 dph exhibited the highest specific growth rate (SGR) and final body weight (FBW) as well as the best Feed Conversion Ratio (FCR) (Table 2). Comparison of the feed performance

Figure 2B. *In vitro* digestibility of four commercial post weaning feeds in European sea bass.



Digestibility rates (%)							
mg dietary protein 5 10 15 30 300							
Feed B	95.4	47.2	40.5	19.2	5.4		
Feed C	77.2	53.9	26.8	22.1	4.3		
Feed D	73.1	34.2	31.8	18.3	4.4		
Feed A	67.6	46.5	30.1	18.4	3.7		

according to SGR, FBW and FCR led to the same rating as with OPTIFeed Technology measurements. B was the top performer followed by C, D and A.

Is trypsin activity a good indicator of larvae performance?

In both feeding trials described above a series of digestive enzymes were screened as indicators of a) the level of maturity of the digestive tract, b) the digestive capacity, and c) the growth performance. Tryspin and chymotrypsin along with carboxypeptidases A and B and leucine aminotransferase were measured. Correlation matrices revealed a consistent significant correlation between trypsin activity and body weight at all developmental stages studied, feeds and feeding protocols (Tables 1 and 2). These findings add to the long-standing discussion on the robustness of trypsin and chymotrypsin activities as growth performance markers.

Concluding remarks

Unlike traditional approaches in Fish Nutrition that rely heavily on time-consuming, costly, qualitative *in vivo* techniques, OPTIFeed Technology has proven a cost-effective quantifiable *in vitro* benchmarking tool. The fish feed industry needs a whole new mentality to face current challenges: formulating diets based upon digestible protein per species and life stage can ensure supply of optimum levels of dietary protein. OPTIFeed Technology can assist the aquaculture industry to move to a new level of optimization moving from Feed Conversion Ratio to the **Economic Feed Conversion Ratio**.

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BERNAQUA biotechnology environment research mutition





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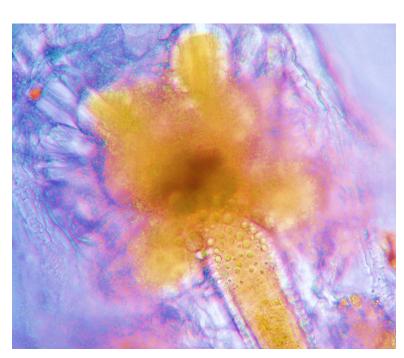
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Today's Challenges Create Tomorrow's Opportunities Through Technically Advanced Feeds and Nutrition

Utilization of advanced hatchery feeds allows operators to take a more proactive and less reactive approach to combating disease. Chris Stock, of Zeigler Bros., Inc. explains.

Shrimp hatcheries, like all sectors of agriculture are subject to a constantly changing set of challenges from the environment, the market, and even "Mother Nature" herself. How each operation reacts to the dynamics of these pressures greatly influences their success and long-term staying power.

Presently acute hepatopancreatic necrosis syndrome (AHPNS) is dominating discussions as it continues to put tremendous pressures on some countries and producers. Of course AHPNS is not the first disease to severely impact the shrimp industry and it certainly won't be the last. While the immediate impacts of disease outbreaks are quite regrettable, opportunity is often disguised within the chaos. AHPNS and other disease epidem-



Lipid vacuoles in the hepatopancreas of a shrimp larva fed Artemia replacement diet, EZ Artemia.

ics have forced even the most reluctant operators to critique their production practices and look for ways to improve. In this article we will assess the opportunities to use nutrition to improve production, reduce exposure to pathogens and limit the risk of loss when disease does strike.

Genetics, environment and nutrition are recognized as the key spheres of influence upon production. Proper management of these factors will significantly reduce both the variability in production and the risk of disease. Of the three aforementioned factors, hatchery and farm operators arguably have greater direct control over nutrition than any other area, yet there is

often considerable resistance to changing feeds and feeding protocols.

Case in point is the persistently high degree of dependence on live or fresh feeds in hatcheries and maturation units. Only under the considerable pressure created by AHPNS does it seem that the topic of bio-security and its connections to feed have received appropriate attention among hatchery operators. Use of live and fresh feeds have long been identified as a key risk factor for introducing pathogens and yet reduced dependence on all live feeds is possible, including complete replacement of Artemia which is increasingly practiced by hatcheries using Zeigler's EZ Artemia.

Table 1: Influence of premium hatchery feeds on complete lifecycle of shrimp (16 pond commercial trial in Central America).

Dramium								
Premium Standard								
4.15mm	3.90mm							
10.14mg	7.14mg							
Phase 2 Hatchery - Same Standard Diets								
Phase 3 Growout - Same Standard Diets								
8	8							
15m ²	15m ²							
0.98gm	0.86gm							
3,630	2,986							
148	153							
53%	47%							
	10.14mg - Same Stand - Same Stand - 8 - 15m² - 0.98gm - 3,630 - 148							

Bio-security risks aside, live feeds fail to provide a reliably consistent nutritional profile. Variation in the source and quality of the purchased cysts or live feed cultures and the differences in hatchery culture environments, the period of harvest, etc. all contribute to differences in nutrient quality and consistency of live feeds when they are finally consumed by the shrimp larvae. And of course the supply of some live feeds, *Artemia* in particular is highly unpredictable, making feeding protocols which are heavily dependent on them at risk for instability when supplies are limited.

While live feeds can introduce disease, high quality, advanced artificial hatchery diets can have the opposite effect, strengthening animals against stressful situations and helping them be more successful at combating pathogenic agents. Unlike live feeds, artificial diets can be used as vehicles to directly deliver health promoting compounds such as probiotics and immune enhancing compounds to developing animals. To better support the health of larval shrimp fed its diets, Zeigler has incorporated Vpak, an all natural nutritional additive designed and proven to enhance an animal's natural immune response to viruses and other pathogens, into all its shrimp hatchery diets. Utilization of advanced hatchery feeds such as these allow operators to take a more proactive and less reactive approach to combating disease.

Broodstock ponds, maturation units and hatcheries are the true headwaters from which all production originates and if animals are not given the best start possible, the implications will carry through every stage downstream. Field studies (Table 1) have shown that when superior larval feeds are provided in the hatchery and compared against controls fed standard hatchery diets, the effects on animal health and performance can be identified long after both sets of animals have been placed in the same grow out conditions and fed the

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same exact grower diets. In essence, at no other time in the production cycle of a shrimp will the quality of the nutrition provided be as influential on long term health and performance as at the hatchery and brood stock stages.

In shrimp farming, just like in any form of agriculture, old habits die hard and many still cling to what they know for fear of change and the unknown. Yet the real risk is often not pursuing new ideas and methods, for those who resist change are often putting themselves at the greatest risk of becoming extinct.

Are you proactively evolving your nutrition program? Does it ensure a constant nutrient profile or is it subject to variation that can put your larvae at a disadvantage? Does your feeding program improve bio-security? Can it proactively improve immune response and ensure the strongest PL's possible? Now is the time to take a look at your hatchery feed protocols and ask yourself these questions. If you answered yes to any of those questions then it's time to consider how you might better use hatchery nutrition to improve your operation.

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ABOUT THE AUTHOR

Chris Stock, MSc. is the Sales Manager - Eastern Hemisphere, Zeigler Bros., Inc. Established in 1935, Zeigler Bros., Inc., is a global pioneer in the field of aquaculture nutrition, hatchery feeds and the application of nutritional immunology to aquatic species.

For more information contact Chris Stock.



A Proven Alternative to Natural Artemia

EZ Artemia has demonstrated higher survivals and animals having well-marked. digestive tracts... we have replaced 100% Artemia in 2011." - Mexico

"We just conducted trials replacing live Artemia in PL transport... we intend to start using it in our larval rearing."

"EZ Artemia can replace 100% Artemia..." – Vietnam





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Nursery feeds for shrimp

Bridging the gap between hatchery and growout

Eric De Muylder, CreveTec, argues that improved biosecurity, better control and a shortened production cycle are just some of the benefits of using a pre-growout system for shrimp between hatchery and growout. A feed designed specially for this crucial stage of production ensures optimal nutrition, growth and survival.

The production of shrimp is generally divided between a hatchery, which produces PL12, and the growout farm where these postlarvae are stocked in bigger ponds untill harvest. During this transfer, the postlarvae are moved from a super intensive system, with total control on water quality, disease prevention or treatment and high quality nutrition to a very extensive system, based on a mixture of phytoplankton and zooplankton.

The success of this transfer highly depends on the quality of postlarvae and the pond condition. Very often, low survival, which can be due to low quality postlarvae or bad condition of the pond, compromises the production of a pond or farm during a complete cycle of 4-6 months.



Quality of postlarvae can considerably, and depend the conditions of the hatchery. Bacterial challenges are often treated with antibiotics, which solve the bacterial problem but don't assist the shrimp's resistance against diseases.

Hatcheries also have different production methods, such as the amount of artemia given, the utilization of microalgae, and the quality of hatchery feeds used. This will higly influence the

Shrimp postlarvae

nutritional status of the postlarvae before selling and consequently the survival and growth in the farm. When these two businesses are completely separated however, the hatchery has no direct benefit from higher quality postlarvae, except for its reputation.

Due to biosecurity, there is a trend towards more intensive farming and lower water exchange. But even in intensive farming the initial growth of postlarvae when stocked in the ponds, depends



PL tanks

completely on the availability of natural feed such as miro-algae and presence of zooplankton (nematodes, copepods, rotiferes, etc), so basically it is initially an extensive system. Utilization of feeds contributes very little to the growth of shrimp during the first weeks, because the pond is too big and feed utilization too low to have enough interaction between shrimp and feed. Basically, the chances of feed being consumed within the first minutes after feeding is really small. Utilisation of high quality (expensive) feeds is therefore not important.

This intensification further increases the importance of good survival rates and final harvest, since overhead costs become more important than variable costs like cost of postlarvae and feed.

Pond preparation before stocking is a critical factor in the production, and can takes up to three weeks of production cycle time. Furthermore, the "production" during the first

CreveTec

POSTLARVAL SHRIMP FEEDS

These postlarval larval feeds have been formulated with high quality ingredients. All proteins sources are hydrolyzed or dried at low temperature for optimal digestibility. The feeds contain micro-algae. They also contain all necessary nutrients for shrimp such as phospholipids, cholesterol and available Phosphate sources.

	L200	PL300	PL500	PL800	PL1000	Broodstock	
For shrimp	M3-PL2	PL3-PL12	PL12-0,5 g	0,5-2,0 g	2-4 g	>30g	
Туре	crumble	crumble	crumble	crumble	crumble	pellet	
Dimensions	100-200 µm	200-300 µm	300-500 µm	500-800 µm	800-1200 µm	2mmx4mm	
Composition:	Crude Protein: 54 %, Lipids: 12 %, HUFA: 1,6 %, Cholesterol: 0,5 %						

Ingredients used: LT Fish meal, Squid meal, Krill hydrolysates, Fish hydrolysates, Wheat products, Lecithin, Fish oil, Hydrolyzed proteins, Micro-algae, Macro-algae, Gelatin binder, Cholesterol, Hydrolyzed Yeast, Vitamins and Minerals.

Packed in alu foil bags of 1 kg or buckets of 10 kg. Tailor-made packing on demand.

Distributors wanted. Please contact:



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month, is very low when calculated as kg/ha*day.

One solution for this is the utilization of a nursery system (or pre-growout) in between hatchery and growout. This improves biosecurity because water input can be treated. It also enables а better control on the nutrition of shrimp, since better quality feeds can be used with a guarantee that they will be consumed by the shrimp. After a couple of weeks, shrimp can be tranferred to the growout pond with the exact count, SO initial mortality can bе compensated.

Such a system will also reduce the production cycle by a month, which increases total production capacity of the farm considerably.

Of course, standard growout feeds will not be adequate for such nurseries, while hatchery feeds are too expensive. It is advisable to utilize high quality feeds, comparable with hatchery feeds, but in bigger sizes.

CreveTec has launched such postlarval feeds.



10 ton nursery tanks



Small pre-growout ponds

These feeds are available for postlavae at the hatchery, but also for nursery stages and up to 2g shrimp.

By utilizing a standard production method the production cost can be controlled.

The focus is instead on nutrition with no compromise on ingredients used.

These feeds contain high quality marine proteins like LT fishmeal, squid meal and krill meal, as well as hydrolyzed vegetable and animal proteins, all proven to be digestible for

shrimp. Apart from this, they also contain micro-algae, which are essential for postlarvae and have been proven to improve growth and survival. Apart from being nutritionally balanced, these feeds are highly digestible, which is essential for postlarvae, which have only a rudimentary gestive system.

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ABOUT THE AUTHOR

For more information, please contact **Eric De Muylder**, Director CreveTec.

YOUR ESSENTIAL GUIDE TO HATCHERY FEEDS

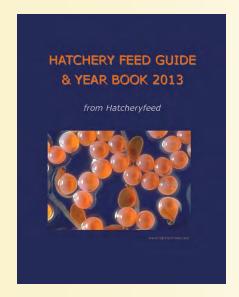


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DIRECTORY

Manufactured feeds

Product listings

Species look up

Water conditioners, enrichments & additives

Product listings

Species look up

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LARVIVA Pro

a big step for the future of fry production

BioMar has launched the first larval diets to include a probiotic approved in the European Union for the use in shrimp and fish feed. First results in commercial hatcheries indicated substantial—and sometimes total—replacement of live feed is possible and demonstrate health, growth and production advantages, writes Irmgard Lorenzen, International Marketing Coordinator, BioMar.

Hatcheries with industrial scale production of fry have always had to put much effort into stabilizing and improving the productive outcome, standardizing production methods and obtaining independence from live prey. But the replacement of live feed in hatcheries seemed unattainable - until recently. Today, the replacement of live feed in fry production is more feasible than ever and the new BioMar product range for hatcheries, LARVIVA Pro, completes the efforts of hatcheries.

"First results with using LARVIVA Pro in commercial hatcheries indicate that it is possible to replace a large percentage of the required live prey to produce sea bream fry. So far, the use of LARVIVA Pro in four commercial hatcheries in the East Mediterranean has resulted in the replacement of a large percentage of rotifers, and in some cases it was even possible to obtain full replacement of the rotifers! And in shrimp farming, the use of LARVIVA Pro Shrimp reduced the need for live feed by more than 50%", said Kostas Ntomalis, in charge of sales and technical support for the hatcheries segment at BioMar, based in Greece.

BioMar has in recent months launched its hatchery feed range LARVIVA Pro. LARVIVA Pro comprises two completely new dry feeds, for fish and shrimp respectively. LARVIVA ProStart and LARVIVA ProWean are the new early weaning diet and weaning diets for fish. The new products for shrimp hatcheries are named LARVIVA Shrimp ProStart and LARVIVA Shrimp PL. These feeds incorporate the latest knowledge about the nutritional needs of the larvae and a major innovation: LARVIVA Pro is the first larval diet to include a probiotic approved in the European Union for the use in shrimp and fish feed for its documented beneficial effects and in particular in reducing deformities in fish larvae and fry.

"In recent years important steps have been made in the Mediterranean area regarding hatchery production techniques, improvement of production facilities and even in genetic selection", said Ntomalis. "The critical role of proteins in the diet of larvae has been well recognized. But despite all efforts to enrich the live feeds with proteins this is almost impossible since the protein contained in the live feed is determined by the genetic code of the animal".

"The easiest and most reliable way to offer the fish larvae all the nutrients that they need for their best development is through dry feeds. The need for replacement of live prey in fry production arises from instabilities in the quality of the live feed, frequent availability problems, high cost of production and biosecurity problems related to the use of live feed. But most of the efforts to replace the live feed have failed or could not provide any quarantee for stable results".

According to Ntomalis, products that were designed for live feed replacements have shown unstable and sometimes contradictory results during the past and feed production companies and several commercial hatcheries do not have the same opinion about the effectiveness of the products offered.

"We looked into this problem", said Ntomalis and continued: "Why could the same products at some hatcheries replace a big part of needed live feed with success and in other hatcheries fail completely? If we isolate the probability of incorrect protocol use, a very



Kostas Ntomalis



Reduces deformities in larvae and fry

LARVIVA ProStart™ is the first early weaning diet with a unique probiotic approved by the European Food Safety Authorities for its documented effect in reducing the occurrence of vertebral deformities in fish larvae and fry.



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important factor that may explain these contradictory results is the bacterial micro flora that is prevailing in the digestive tract of larvae living in different hatcheries or even living in different batches of the same hatchery. This micro-flora of the digestive tract significantly affects the digestibility of feed and the level of absorption of nutrients, among other things. And it may even cause inflammation of the digestive tract of the larvae. There are several examples. And unfortunately we have found rotifers not just undigested but even surviving and living in the digestive track of sea bream larvae!"

According to Ntomalis the above is an indication that the capability of these larvae to digest has decreased significantly. "Usually this is not due to bad quality or low digestibility of live feeds. A large number of scientific work is directly linking the survival and quality of larvae and fry with the microflora in digestive tracts, and especially with the presence of *Vibrio anguillarum* derived from live feeds, mainly from rotifers", said Ntomalis. "Other studies have identified a sharp increase of the bacterial load in the digestive tract of the larvae when they are taking the first live feed. It goes without saying that this sudden change strongly affects the survival of larvae and consequently the quality of the fry, especially at such a sensitive stage of development".

The disadvantages of use of live feed in production of fry have been recognized by BioMar and in recent years BioMar has been working methodically in order to be able to eliminate them.

"With the new product line LARVIVA Pro containing the probiotic strain *Pediococcus acidilactici MA18/5M* the control of microflora in the digestive tracts is feasible and in this way we have made an important and pre-requisite step towards reliable supplementation or even complete replace of live feed in future", said Ntomalis. BioMar is confident that the control of microflora in the digestive tract leads to improved and more predictable production results and that the sooner this is done in larvae the better will be the outcome.



Deformities in sea bream and sea bass fry engender significant economic losses in Mediterranean fish farming.

"If you want to succeed consistently and to have reliable results in the complement or replacement of live feed it is obvious that it is important to control the microflora in the digestive tracts. But the physical characteristics and the protocol of use should also be appropriate", explained Ntomalis. "Although to date we have very positive results in many commercial hatcheries that have used LARVIVA ProStart, especially in sea bream, we recommend to our customers to be cautious. The product is brand new and customers have to fine-tune their own feeding protocol to get the best results. Changes in production facilities may be required, like increasing the potential renewal of the tank water. Significant changes in the production protocol may also be required, for instance one should consider adapting the enrichment of live feed. This is due to the fact that most of the enrichment products are not designed to be used simultaneously with dry feeds into larvae, and some of the products may be the cause of slow growth or even of serious malformations".

"Successful rearing of fish and shrimp depend on the production of more predictable and high performance fry", Ntomalis said.

"We consider LARVIVA Pro to be an important step forward in that direction. We are now working together with hatcheries to implement our new products to an even greater extent in the production of fry and to adapt them to the local conditions of each hatchery", he explained.

"The response coming from the hatcheries is very positive and we are grateful to know that customers for sharing their experiences with us, as the further development of LARVIVA Pro depends on this cooperation".

For more information, contact Kostas Ntomalis, BioMar Greece



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From Start to Smolt with EWOS

EWOS juvenile feeds for salmon have an excellent reputation in the market place in all salmon growing countries. The success of these feeds is attributed to the control of important factors and innovations in research and development for this critical part of the salmon life cycle. Louise Buttle PhD. and Ian Carr, EWOS Group explain.

Juvenile feed performance is defined by nutritional factors and physical factors. Nutritional factors include macronutrient levels such as energy, protein and fat and also micro-nutrient levels such as the levels of minerals and vitamins in the feeds. Physical factors mainly include particle size distribution, minimal dust levels, water stability and sinking speed.



EWOS Innovation, Dirdal, Norway

Nutritional Factors

Internal reviews of the nutritional requirements of salmon are routinely performed by EWOS through research trials. However, in the spirit of open innovation, third party research is also reviewed. For example, The National Research Council (2011) recently published material on the Nutrient Requirements of fish and shrimp. This is an excellent reference, as many of the studies are done on small fish.

With a good understanding of the nutritional requirements of juvenile salmon, the next step is to understand the nutritional profile of the raw materials. EWOS Innovation research shows that raw material selection is important in all feed stages, but this is especially true for starter feeds. A large percentage of the raw material in starter feeds is fish meal. We know that fish meal can vary in quality between suppliers, depending on the source species, treatment of the raw material and several other factors. We also know that changes in fish meal quality

may affect fish performance. Because of this, EWOS Innovation has a routine raw material screening of global fish meal types using advanced chemical techniques as well as rapid fish trials. Many other raw materials category types are also screened. In 2012 EWOS Innovation screened almost 100 raw materials. Accordingly, several EWOS inhouse advanced tools have been developed to check quality and consistency of raw materials used in juvenile feeds. These tools enable EWOS to ensure that the best quality fish meal is selected for EWOS starter feeds for optimal and consistent performance of this small size feed pellet.

Benchmarking Trials

How do EWOS know that performance is optimal and consistent? Annual starter feed benchmarking trials assess the performance of EWOS starter feeds against competitor diets in the market place as part of EWOS's research program. Results from these comparative studies are important in establishing if improvements and refinements in dietary formulations are needed.

The use of functional feed ingredients has been a key factor in the optimal and consistent performance of EWOS starter feeds. EWOS has a long history of using a nucleotide preparation giving optimum fish performance and a good immune status in the fish.

Physical Factors

Excellent nutrition must be delivered in a perfect package! The effect of feed size at first feeding has been a recent focus of R & D in EWOS. Studies found that at a larger feed size there was a small benefit on fish performance – although at start feeding weights of less than 0.15 g caution should be exercised. Customer preferences and experience are very important in the early life stages of salmon. For example, it is important to consider the type of production system in use, when selecting feed.



In recent years, there has been a rise in interest in farming salmon in recirculation aquaculture systems (RAS). For example, in Chile about 30% of Atlantic salmon smolt facilities are RAS and it is predicted that there could be approximately 85 million smolts produced in RAS in Norway by 2015 (Matius del Camp, 2010). This increasing use of RAS technology increases the focus of all aspects related to feed quality, raw material use and the particle size and stability of the fish faeces. In this way, EWOS has had a recent focus on recirculation feeds and current guidelines include a focus on the technical quality of the feed, the selection of highly digestible raw materials, good binder sources and bioavailable mineral sources. In addition, the feeding management of RAS systems should

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be focussed on minimum uneaten feed - and "even" feeding throughout the day. The screening of novel raw materials and applications for RAS feeds has also been the focus of research and development in EWOS Innovation. As well as the digestibility and physical quality of the feeds, the physical properties of the faeces was a key indicator in the research trials.

Knowledge makes the difference

Globally, EWOS is one of the main suppliers in the freshwater market. Many of the customers want a supplier that can help with advise in the production and make sure that the feed is being used the best way. EWOS has made feeding tables for start feeding, and this helps the customer to make good plans and have a reference to follow in the production. Some of the largest hatcheries in Norway for example produce over 10 million smolt a year, and



then the feed must perform optimally to make sure that everything goes as planned.

EWOS has built its reputation upon supplying well researched and documented feed and nutrition for aquaculture. This applies to juvenile feeds as well as to feed for larger sizes of fish. Proof of performance comes through satisfied customers who depend upon EWOS for their juvenile feed from year to year.

EWOS is a global feed company operating in five countries (Canada, Chile, Norway Scotland and Vietnam). The main feed business is salmon aquaculture. In 2012, EWOS produced over 1 million tonnes of salmon feed.

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