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Compound microdiets for marine fish larvae: Key parameters

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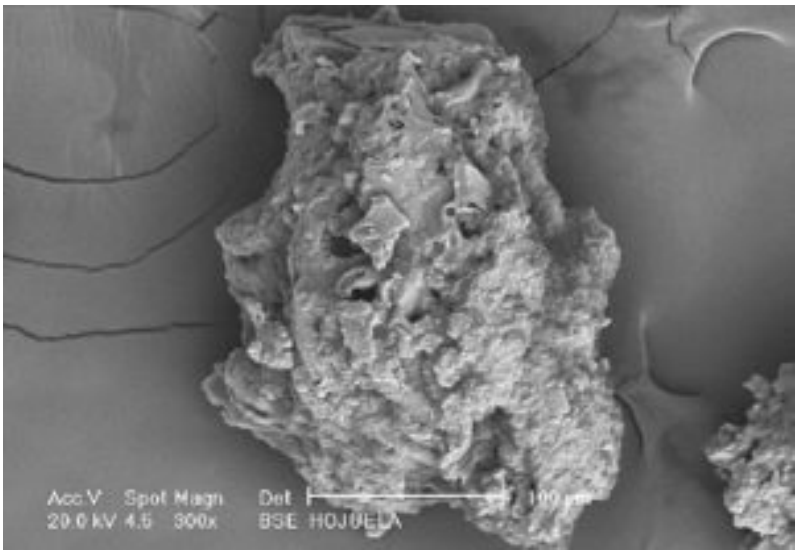
Authors recommend diet stability and palatability assays



Scanning electron microscopy photograph of a microdiet.

Production of live food for the rearing of larval marine fish constitutes a major operational cost and bottleneck in commercial marine fish culture. The variability and poor nutritional quality of live food are major concerns during production.

Research has been directed toward developing compound microdiets to replace live food to ameliorate this problem. Although extensive research has been performed during the last 25 years, studies to determine specific nutritional requirements such as amino acid and vitamin requirements are scarce and the formulation of adequate diets is still elusive.

Formulating complete diets

Larval performance on compound microdiets alone is, in general, inferior to that of live food. One question that arises from previous studies is whether the technological processes utilized to manufacture microdiets is adequate to emulate live food acceptability and digestibility. The authors recommend that diet research incorporate comprehensive evaluations that include diet stability and palatability assays, which are not usually performed for marine larvae diets.

Much work has been devoted to biological evaluation, such as bioassays that last longer than six weeks, but the only parameters evaluated in most feeding trials are growth and survival. The authors propose that several key parameters be investigated during the manufacturing of practical compound microdiets for first-feeding marine fish larvae.

Research process

The ideal compositions of compound microdiets are species-dependent, but common guidelines can be followed based on previous research (Table 1). The authors have modified the approach suggested by Yufera and coauthors in 2000, which included a three-step process: diet design, biological evaluation and digestibility evaluation. The following research strategy is proposed.

- Selection of ingredients.Use fresh by-products from marine organism processing plants (although they represent potential vectors for disease), fishmeal, and a protein hydrolyzate to provide intact proteins, peptides, and free amino acids, as well as highly unsaturated fatty acids (Table 2).
- Selection of binder/technology. Use a mixture of polysaccharides and proteins that are highly digestible. The application of combing fluidized bed dryer and microbound flake technology is suggested.
- Stability evaluation. Test the leaching rate of soluble protein, peptides, and/or amino acids. The authors evaluated soluble protein using a modified Bradford technique.
- Acceptability evaluation. Study ingestion rates with and without gentle aeration during five hours using larvae not fed for 24 hours. The authors evaluated animals at 16 days posthatch.
- *In vitro* digestibility. Compute using the following equation.
  Relative protein digestibility = (Δ Abs.280_{CMD} / Δ Abs.$280_{Caseine}$) x 100

Medina-Reyna, Recommended nutrient levels for compound microdiets, Table 1

Nutrient	g/100 g diet Minimum	g/100 g diet Maximum
Proteins	50	65
Free amino acids		10
Peptides		20
Intact protein	30	
Lipids	10	20
Triglycerides		5
Phospholipids	10	
22:6n-3	2	
20:5n-3	1	
20:4n-6	0.1	

Nutrient	g/100 g diet Minimum	g/100 g diet Maximum
DHA:EPA	2	
EPE:ARA	5	10
HUFAs n-3	3	5
Carbohydrates		10
Fiber		2
Ash		10
Vitamins	5	
Ascorbic acid	0.5	
Minerals	4	
Attractants	2	4

Table 1. Recommended nutrient levels for compound microdiets for marine fish larvae.

Medina-Reyna, Experimental diet formulation, Table 2

Ingredient	g/100 g diet
Menhaden fishmeal	49.4
CPSP 90	14.8
Skipjack muscle	9.8
Skipjack orbital oil	14.8
Cod liver oil	4.9
Carrageenan	0.62
Mesquite gum	3.1
Sodium alginate	0.6
Whey protein concentrate	0.6
Vitamin premix	0.1
Mineral premix	0.2
Proximate composition (%)	
Protein	67.4
Lipid	21.3
Dry matter	93

Table 2. Experimental diet formulation.

Experimental diet tested

In a study that applied the above stategy, the authors selected several ingredients available locally and combined them with fishmeal to provide a nutritionally balanced diet for marine fish larvae. Although the 67 percent protein content seems high, previous experience has shown lower ingestion rates with microdiets. The level was chosen to satisfy requirements using a nutrient-dense approach.

A binder mixture enhanced the mechanical properties of the dough during the flaking process, but pieces of fishmeal were not hydrated satisfactorily or homogenized as fresh ingredients. The combination of these factors resulted in a diet that was easy to prepare.

Although the ingredients were finely ground to 10-20 µ to produce a microdiet of 250 µ, the soluble protein-leaching rate was a satisfactory 0.5 percent per hour. Leaching of other nutrients should be evaluated to provide a more comprehensive evaluation of diet stability. The relative protein digestibility of 80.4 percent seemed adequate, but should be confirmed using larval digestive enzymes and ultimately correlated with *in vivo* assays.

Acceptability tests with California halibut larvae were initially performed in tanks without aeration. The compound microdiet tended to sink quickly in less than 30 minutes. When gentle aeration was provided, ingestion rates significantly increased, with even higher rates in the presence of microalgae. The behavior of the diet in the water column must be evaluated and optimized prior to biological evaluation trials.

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