

Development and Characterization of Several Open Formula Reference Diets for Marine Fish Larvae

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Abstract: A limiting constraint in the development and growth of marine aquaculture is successful hatchery production. Presently, larval finfish are raised using live feeds such as copepods, rotifers and *Artemia*. Live feeds are expensive, time-consuming, labor intensive, unreliable and nutritionally imperfect. An alternative to live feeds is microparticulate feeds, however, high performance microparticulate diets for larval finfish are typically closed formula commercial diets that make scientific inference difficult or impossible. We describe a series of open formula microparticulate reference diets that have been formulated and produced to facilitate comparisons across species and systems for use by the scientific community. Open formula diets can produce consistent results among trials and species, they can be simply formulated with well-defined ingredients and provide a basic platform for improvement. The reference diets were processed by three methods: flaking (F), microextrusion followed by marumerization (MEM) and particle-assisted rotational agglomeration (PARA). An additional two diets were made by using the flake diet and further processing it using the MEM (F-MEM) or PARA (F-PARA) methods. The F, F-PARA and F-MEM diets had the same formulation and only differed by processing method. PARA and MEM had unique formulations. Each diet was screened to the 400–700 µm range. All microparticulate diets were compared to enriched *Artemia* and rotifers (*Brachionus plicatilis*) for chemical analysis. Scanning electron microscopy (SEM) was used to visualize the microparticles between 22x and 4000x magnification. It was clear that micropellet structure, sinking rate and leaching was influenced by both formulation and manufacture method. Basic structure remained largely intact for all but the F-PARA particles even after being immersed in fresh water for 15 minutes. Compositional data, leaching half-life and sinking rates are given in the table below (values in a column with differences at P<0.05 are denoted by superscripts). Data on feeding trials with larval fish indicate that two of the open formula diets performed as well as, or almost as well as, a popular commercial diet (Otohime, Marubeni Nisshin Feed, Tokyo, Japan) with larval yellowtail (*Seriola lalandi*), white sea bass (*Atractoscion nobilis*) Pompano (*Trachinotus carolinus*) and red drum (*Sciaenops ocellatus*). This performance illustrates that these diets provides a good base on which to make improvements.

Annotated Bibliography

- Barrows F. T., and Lellis W. A., 2000: Microbound feeds. In R. R. Stickney (ed) Encyclopedia of Aquaculture. John Wiley & Sons, New York, 525–528.
- In addition to a review of methods to make older micro bound feeds for aquaculture, methods to

2015年1月30日受理 (Received on January 30, 2015)

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produce microparticulate feeds using micro extrusion and particle assisted rotational agglomeration are explained.

Cho C. Y., Cowey C. B., and Watanabe T., 1985. *Finfish Nutrition in Asia: Methodological approaches to research and development*. IDRC, Ottawa, Ont. Canada. 154 p.

Step by step instructions to produce zein and carrageenan bound diets developed in Japan are explained in English.

Holt G. J. (editor) 2010: *Larval Fish Nutrition*. John Wiley & Sons, New York. 435 p.

Nutrition is particularly important in the healthy development of fish during their early-life stages. Understanding the unique nutritional needs of larval fish can improve the efficiency and quality of fish reared in a culture setting. *Larval Fish Nutrition* comprehensively explores the nutritional requirements, developmental physiology, and feeding and weaning strategies that will allow aquaculture researchers and professionals to develop and implement improved culture practices. *Larval Fish Nutrition* is divided into three sections. The first section looks at the role of specific nutrient requirements in the healthy digestive development of fish. The second section looks at the impacts of nutritional physiology on fish through several early-life stages. The final section looks at feeding behaviors and the benefits and drawbacks to both live feed and microparticulate diets in developing fish.

Langdon C. L., 2003: Microparticle types for delivery of nutrients to marine fish larvae. *Aquaculture*, **227**, 259-275.

Good review and discussion of several types of microparticulate diets including complex microparticles.

Rønnestad I., Yufera M., Ueberschär B., Ribeiro L., Sæle O., and Boglione C., 2013: Feeding behaviour and digestive physiology in larval fish: Current knowledge and gaps and bottlenecks in research. *Rev. Aquaculture (suppl. 1)*, S59-S98.

Food uptake follows rules defined by feeding

behaviour that determines the kind and quantity of food ingested by fish larvae as well as how live prey and food particles are detected, captured and ingested. Feeding success depends on the progressive development of anatomical characteristics and physiological functions and on the availability of suitable food items throughout larval development. The fish larval stages present eco-morpho-physiological features very different from adults and differ from one species to another. The organoleptic properties, dimensions, detectability, movements characteristics and buoyancy of food items are all crucial features that should be considered, but is often ignored, in feeding regimes. Ontogenetic changes in digestive function lead to limitations in the ability to process certain feedstuffs. There is still a lack of knowledge about the digestion and absorption of various nutrients and about the ontogeny of basic physiological mechanisms in fish larvae, including how they are affected by genetic, dietary and environmental factors. The neural and hormonal regulation of the digestive process and of appetite is critical for optimizing digestion. These processes are still poorly described in fish larvae and attempts to develop optimal feeding regimes are often still on a 'trial and error' basis. A holistic understanding of feeding ecology and digestive functions is important for designing diets for fish larvae and the adaptation of rearing conditions to meet requirements for the best presentation of prey and microdiets, and their optimal ingestion, digestion and absorption. More research that targets gaps in our knowledge should advance larval rearing.

Feed	Moisture (%as fed)	Proximate Composition			Fatty Acid Concentrations			Protein Leaching $t_{1/2}$ /% lost (sec)/(%)	Sinking Rate (cm/sec)
		(% dry weight)			(mg fatty acid/100mg total fatty acids)				
		Protein	Lipid	Ash	ARA (n-6)	EPA (n-3)	DHA (n-3)		
MEM	5.7	53.8	30.5	8.4	0.4	7.5	5.7	98/23.0	0.84±0.010 ^a
PARA	5.3	54.2	29.7	8.9	0.4	7.4	5.6	94/30.2	0.34±0.001 ^c
F	9.0	75.9	10.9	7.3	1.0	6.0	15.2	20/44.3	0.33±0.001 ^c
F-MEM	13.3	75.6	13.4	7.5	1.0	5.3	13.0	50/41.8	0.78±0.060 ^a
F-PARA	11.0	75.6	12.6	7.3	1.0	6.0	15.2	33/47.2	0.61±0.001 ^b
Artemia	91.3	NA	15.3	13.7	5.6	12.1	7.1	NA/0.1	NA
Rotifers	90.1	49.6	15.5	17.7	1.5	5.7	23.3	NA	NA