Aquafeeds

Artificial microparticles for delivery of nutrients to marine suspension-feeders

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By Chris Langdon

Why are inexpensive artificial feeds not available for the complete replacement of live feeds?

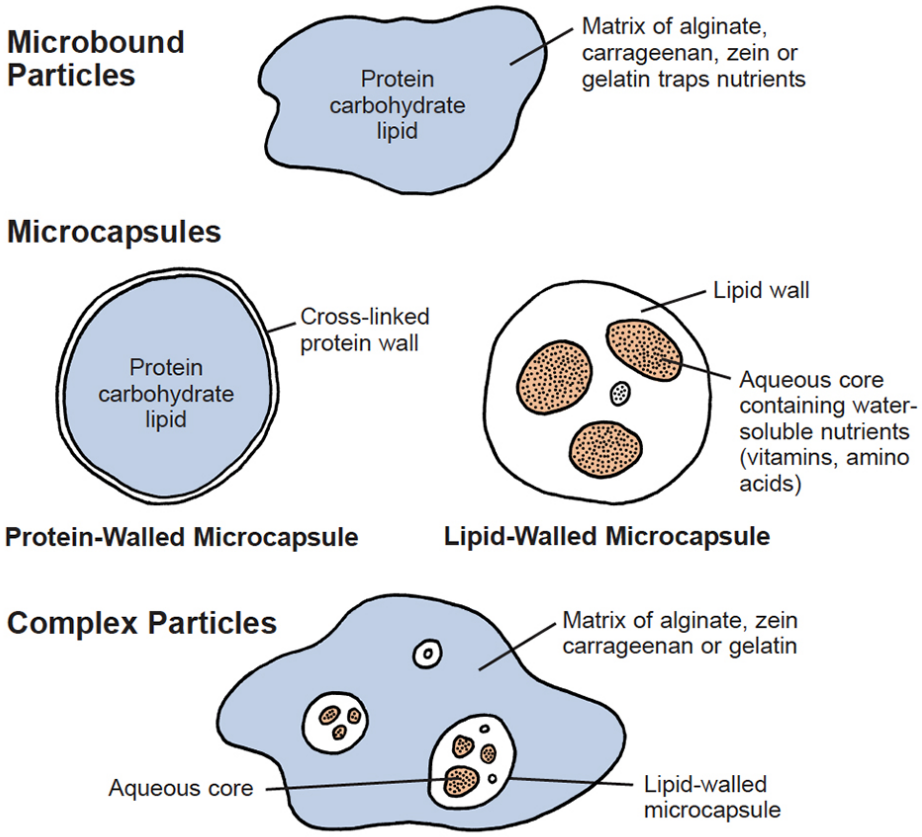

Fig. 1: Microparticle types for delivery of nutrients to suspension feeders.

Hatchery production of the larval stages of marine fish, crustaceans, as well as all the life stages of bivalve mollusks, usually depends on live feeds such as phytoplankton, rotifers, and artemia. Live feeds are expensive to produce and are not always of consistent and dependable quality. Why are inexpensive artificial feeds not available for the complete replacement of live feeds?

The main problems in the development of inexpensive, artificial feeds for suspension feeders have been related to diet delivery rather than diet composition. Small particles (less than 1 mm in diameter) have large surfacearea to volume ratios. Losses of amino acids, water-soluble vitamins and other low molecular weight, water-soluble nutrients are rapid due to high diffusion rates and short diffusion paths through the particle binding material or capsule wall. Researchers have shown, for example, that more than 80 percent of amino acids are lost from alginate- bound microparticles after suspension in water for two minutes.

There have been many attempts to reduce losses of dietary ingredients from microparticles. The most common approach is to bind dietary ingredients in a gel matrix of alginate, carrageenan, zein, or gelatin to produce “microbound” particles (Fig. 1). These dietary particle types are commercially available in powder form. A recent innovation is the commercial production of microbound particles in a slurry or “liquid diet” that can be easily dispersed in the culture medium. Additional ingredients can be added to the liquid diet, such as probiotic bacteria, preservatives and lipid emulsions.

Microencapsulated diets for marine suspension feeders were developed over 20 years ago. The

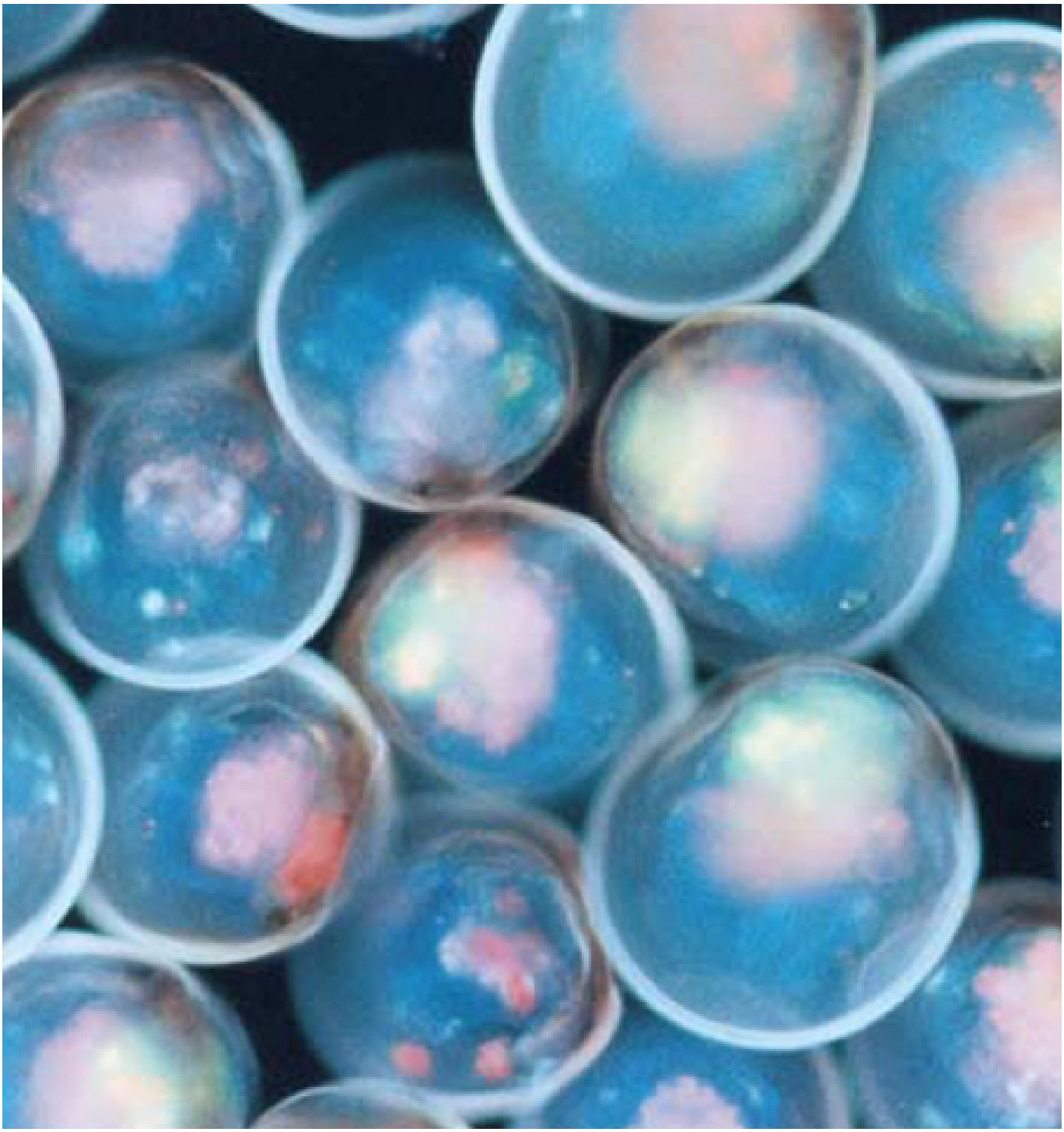

Fig. 2: Eight day-old larvae of the Pacific oyster (*Crassostrea gigas*) viewed under epifluorescent illumination (excitation 355 to 425 nm, emission 525 nm). Larvae were fed on riboflavin containing lipid beads (50 beads/µl) for one hour, followed by a two hour period of feeding on Isochrysis galbana (T-ISO) alone. Free riboflavin was evident as a diffuse greenish fluorescence in the guts of larvae while riboflavin crystals present in intact or partially digested SB were evident as bright yellow points. The digestive systems of some larvae also fluoresced red due to the presence of chlorophyll from ingested algae. Average larval shell length = 122 µm.

advantage of this approach is that dietary ingredients are surrounded within a capsule wall that may reduce leakage rates of water-soluble nutrients. Commercial production of encapsulated diets is often expensive because the manufacture process is more complicated than for production of microbound particles. The walls of most capsule types also are permeable to low-molecular weight, watersoluble nutrients.

Retention of water-soluble vitamins and amino acids by dietary microparticles can be improved by encapsulating these ingredients in lipid (Fig. 2). Lipid-walled microcapsules consist of a lipid wall surrounding an aqueous core. Water-soluble nutrients can be dissolved in the aqueous core and their leakage rates reduced by the surrounding lipid microcapsule walls. Penaeid shrimp larvae are able to crush capsule walls made of solid tripalmitin with their formidable spiny mouth parts and digestive system. In contrast, marine fish larvae and bivalves require softer-walled lipid capsules in order to break down the walls to release dietary contents. Capsule walls can be softened by adding marine fish oils to tripalmitin, resulting in capsules that supply essential polyunsaturated fatty acids as well as encapsulated water-soluble nutrients.

Recently, lipid spray-beads have been developed that consist of particulate nutrients embedded in lipid spheres. The beads can be prepared by spraying the lipid-nutrient mixture into a cool chamber to harden the lipid, allowing the beads to be collected and stored. The advantage of this method is that higher concentrations of core material can be encapsulated and the preparation process is simpler than for lipidwalled microcapsules.

It is likely that the future microparticles for the effective delivery of all dietary nutrients to marine suspension feeders will consist of lipid beads or capsules containing water-soluble nutrients that are themselves embedded in microbound or microcapsule dietary particles.

This combination of particle types is called a “complex” microparticle (Figs. 3 and 4). Feeding experiments with penaeid shrimp larvae indicate that complex particles can be ingested and dietary contents released in the digestive system. Commercial development of such complex particles should greatly reduce reliance on live feeds and lead to less expensive, dependable, high quality artificial diets for marine suspension-feeders.

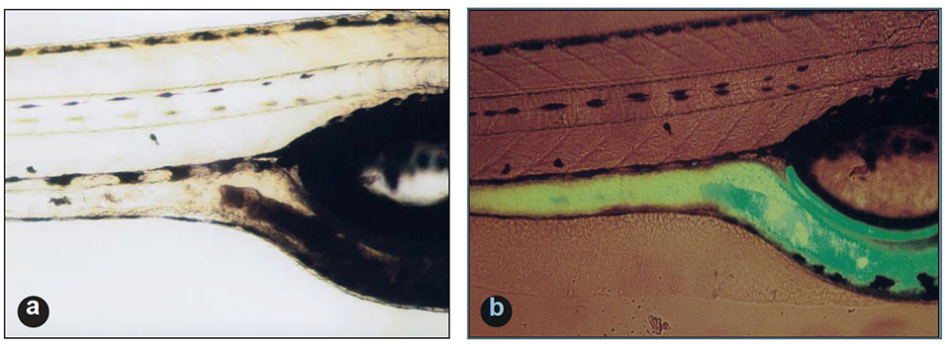

Fig. 4: Stomach and hind gut of a seven day-old, first feeding

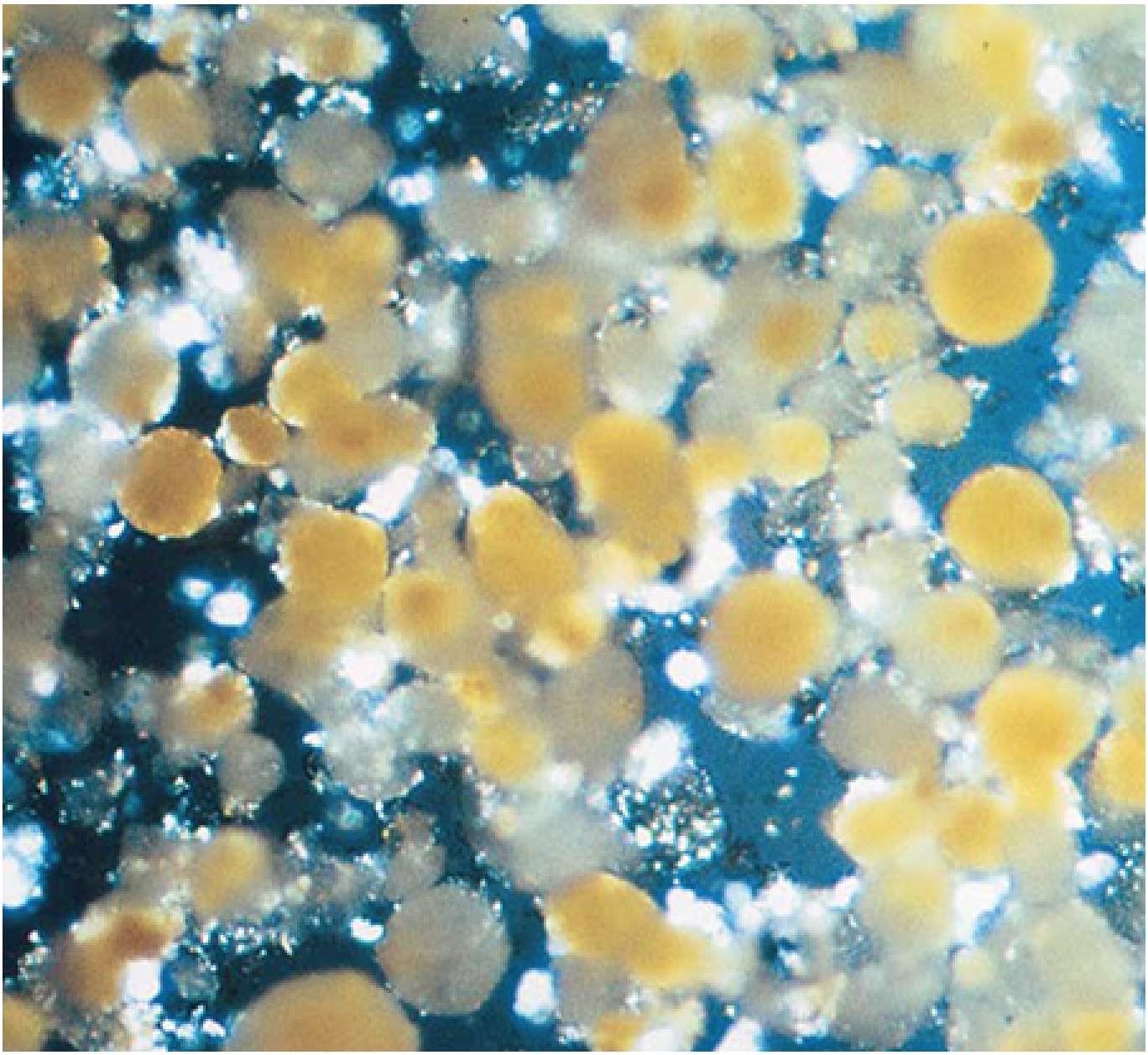

Fig. 3: Complex microparticles consisting of lipid beads containing riboflavin that were themselves embedded in zein microbound particles. Particles ranged from 50 to 150 microns in diameter. Photo by U. Önal.

Zebrafish (*Brachydanio rerio*) larva fed for eight hours on zein complex particles with embedded riboflavin-containing lipid beads (shown in Fig. 3). Photos by U. Önal. 4a. Larval stomach and hind gut under normal illumination, showing a food bolus derived from ingested complex particles entering the hind gut from the stomach. Scale: 1 cm = 120 microns. 4b. The same part of a larval stomach and hind gut shown in Fig. 4a except under epifluorescent illumination (excitation 355-425 nm, emission 525 nm). Riboflavin fluoresced

green or yellow in association with the food bolus while free riboflavin, not associated with the food bolus, was evident as a diffuse yellow fluorescence in the hindgut. Bright yellowfluorescing points in the food bolus may indicate undigested, riboflavin-containing lipid beads. Scale: 1 cm = 140 microns.

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