

Development, testing of microencapsulated Schizochytrium feeds for bivalve broodstock

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European flat oysters show improved sexual development, higher omega-3 levels compared to traditional live algal diets



This research compared the performance of European flat oyster broodstock fed novel microencapsulated Schizochytrium feed and conventional live algae diets; results showed improved sexual development, significantly higher omega-3 levels with the novel diet compared to traditional live algal diets, and potential for use in commercial bivalve hatcheries. Photo by Heigeheige, CC BY-SA 4.0, via Wikimedia Commons.

The U.S. \$17.2 billion global bivalve shellfish industry relies upon a supply of juvenile bivalves produced by adult broodstock in hatcheries. Current estimates suggest 220 million broodstock bivalves are held in hatcheries worldwide, and these animals must be fed live algae. Algal production in hatcheries makes highly inefficient use of land and energy. Typical hatcheries require 400 square meters of algal tanks to maintain just 400 broodstock, equating to 220 million square meters worldwide, an area larger than Washington, D.C. And high-intensity artificial lighting, temperature and air control systems are needed to support algal growth.

Furthermore, algal stocks are difficult to maintain and frequently are lost due to contamination and disease, so larger quantities must be produced, to the extent that each unit of viable hatchery-grown algae is 20-fold more expensive than units grown in contamination-free commercial photobioreactor facilities, accounting for 50 percent of bivalve production costs at \$220 per kg of algal biomass in 2016. And algal production presents a major disease control issue, with live algal feeds as a primary vector of bivalve disease.

New advances in algal production and microencapsulation technology offer a groundbreaking solution to reduce the environmental footprint of bivalve aquaculture. The microalgae *Schizochytrium* can be grown on industrial scales for \$1.50 per kg, using low-cost food waste and agricultural side-streams as inputs. For bivalve nutrition, *Schizochytrium* offers key nutrients such as docosahexaenoic acid (DHA) exceeding 20 percent dry weight, over twice the abundance of DHA in hatchery-grown algae. Mass production is simple and cost-effective, and the microcapsules dry and shelf-stable, thus circumventing conventional feed wastage costs. Microcapsule characteristics can be tailored to maximize feeding efficiency and minimize nutrient leaching to water, while also being sterile and not a disease vector.

This article – adapted and summarized from the <u>original publication (https://doi.org/10.1038/s41598-020-69645-0)</u> (Willer, D.F. et al. 2020. Microencapsulated algal feeds as a sustainable replacement diet for broodstock in commercial bivalve aquaculture. *Sci. Rep.* 10, 12577) – details the development and testing of a microencapsulated diet from the microalgae *Schizochytrium* with European flat oyster (*Ostrea edulis*) broodstock.

Study setup

Lipid-walled microcapsules containing 50 percent powdered *Schizochytrium* algae by weight were manufactured under patent by BioBullets (BioBullets Ltd, Cambridge, UK). All components of the formulation were food grade. The final microcapsules had a diameter between 20 and 140 μ m, spherical shape, and had near neutral buoyancy.

Conditioning experiments on *O. edulis* broodstock were carried out under commercial production conditions at SeaSalter Shellfish (Whistable) Ltd. in Kent, England. The experiments took place over six weeks using three flow-through tanks kept at ambient hatchery temperatures (18 to 24 degrees-C) and salinities (26 to 28 ppt). Each tank contained 15 *O. edulis* broodstock and received one of the following three diets: live algae (SeaSalter's formulation), microcapsules (BioBullets) or algae + microcapsules.

Each feed was fed daily at 3 percent dry weight of feed per mean gram dry weight of broodstock, so oysters on the algae + microcapsules diet received twice as much food as oysters on the single food diets. Feed was delivered using a continuous system with a flow-through rate of 10 ml per minute. At the end of the six-week conditioning period, all broodstock were frozen and transported to the Department of Zoology, University of Cambridge, England, where they were processed for analysis. Before the conditioning period began an additional sample of 15 broodstock was also transported to Cambridge and also frozen for use as pre-conditioning controls.

For detailed information on the experimental design and setup; animal husbandry; collection of oyster gonad samples; fatty acid and lipid analyses; sexual maturation analyses; and data processing and statistical analyses, refer to the original publication.

Results and discussion

There are major sustainability advantages for replacing live algal feeds with microencapsulated feeds in bivalve aquaculture, including 20-fold reductions in energy use, carbon emissions and production costs. However, for replacement to be commercially viable it is critical to assess whether microencapsulated feeds provide comparable sexual development in bivalve broodstock compared to conventional algal feeds.

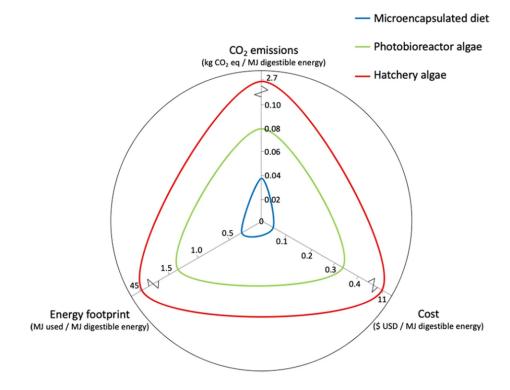


Fig. 1: The sustainability advantage of new microencapsulated diets. The radar-plot demonstrates lower carbon dioxide emissions, reduced energy usage and more efficient use of economic resources in the new microencapsulated diets. Comparison is relative to the most efficient to produce form of autotrophic live algae grown on an industrial scale (photobioreactor algae), and algae grown in a relatively efficient bivalve hatchery today. Note the broken axes for hatchery algae. We also note that a 100 percent replacement of live algae will require further tailoring of the microcapsule formulation for increased protein content. Data sources available in the original publication.

Our investigations demonstrated that *Schizochytrium*-based microencapsulated diets enabled not only comparable but improved sexual maturation in *O. edulis* broodstock compared with conventional live algal diets. The gonads of oysters fed microencapsulated diets had greater weight, contained higher levels of omega-3 fatty acids crucial for sexual maturation, and underwent accelerated spermatogenesis [process of development of the sperm cells within the male reproductive organs].

Of particular importance are the large increases in the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in microcapsule-fed relative to algae-fed oysters. EPA is the primary energy source for gamete maturation, with higher levels directly increasing gamete quantity and development rate. DHA is pivotal to the structure and function of gamete cell membranes, with higher levels increasing gamete quality and egg survival rates. High levels of EPA and DHA in the *Schizochytrium*-based microcapsules are likely driving this increase.

To date there is no clear evidence that either fatty acid can be synthesized de novo (new) by oysters from shorter chain precursors. The more rapid advance of spermatogenesis in oysters fed a microencapsulated diet is likely being driven by the greater levels of EPA and DHA in these animals, a

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causal relationship demonstrated and supported by several previous studies. This offers strong support for the use of microcapsules as a broodstock conditioning feed.

Fig. 2: Increased oyster broodstock gonad weight following conditioning. The boxplot shows the total wet gonad weight of oysters fed algae (green), microcapsules (orange), or algae + microcapsules (purple) over a six-week conditioning period, relative to oysters pre-conditioning (blue). N = 9 oysters per

diet. Gonad weight was significantly greater in oysters fed a diet relative to pre-conditioning of the animals.

It is important to consider that, for future application, the nutritional formulation of the microcapsules would need to be tailored further for increased protein content or fed alongside a quantity of live algae. The current protein content of the microcapsules is lower than that of live algae (6 vs. 31 grams protein per 100 grams dry weight). Protein is important in bivalve larval development and for shell formation, and if insufficient, juvenile growth can be suppressed. There would be significant value in performing additional studies investigating the effectiveness of a higher protein formulation of microcapsules on bivalve broodstock conditioning and juvenile development.

Regarding the newly developed microcapsules, their size is tailored to maximize bivalve feeding efficiency (20 to 140 µm diameter), and buoyancy neutral to ensure particles remain within reach of the filter feeders. This is an improvement over a basic, freeze-dried, algal powder delivery system; powders tend to float on the water surface and can clump into particles too large to be accessed by bivalves. The specialized coating allows delivery of low molecular weight, water soluble compounds, alongside fatty acids, with minimal leaching to the surrounding water, reducing eutrophication risks. The encapsulant also has strong antibacterial properties, and contents are sterile, which reduce disease incidence in aquaculture relative to live feeds.

Improvement of the physical characteristics of the microcapsules is possible. For example, while we know that capsules of 20 to 140 μ m can be ingested by oysters, it would be useful to perform a study to investigate the filtration and ingestion of different sizes of microcapsule within this range to identify the preferred specific capsule diameters for both bivalve broodstock and juveniles.

Regarding sustainability and commercial implications, the use of live algae requires the use of significant resources and energy use in bivalve production. Our research demonstrated that sustainable *Schizochytrium*-based microencapsulated diets can help support more effective sexual maturation in oyster broodstock. Further research could help open up the opportunity to upscale production and associated infrastructure to allow microencapsulated diets to partially or fully replace live algae in hatcheries.

However, given that microencapsulated diets enabled not only comparable but improved sexual development in bivalve broodstock, there is potential to reap even further commercial and sustainability benefits. Higher quality broodstock with greater lipid stores directly translates into higher quality seed with a greater inherent survival rate. More rapid sexual maturation enables seed production earlier in the season, giving the seed a longer growing period prior to their first overwintering, with corresponding greater size and cold tolerance increasing survival.

Increased sexual maturation rates also mean shorter conditioning cycles and greater larvae production for a given hatchery each year, increasing the total output of bivalve seed. As the supply of bivalve seed is one of the biggest factors limiting the growth of the bivalve industry, with demand far outstripping supply, microencapsulated feeds could play an important role in enabling the bivalve industry to expand. Key next steps towards reaching this goal will include further tailoring of the microcapsule formation for increased protein content and use-specific size profile to maximize ingestion efficiency and bivalve growth.

Perspectives

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Bivalve aquaculture is more environmentally sustainable than other forms of aquaculture and meat production, and even some cereal crops. For example, for every new ton of protein that is produced from bivalves instead of fish aquaculture, we spare 9 hectares of land, 67 tons carbon dioxide emissions, and 40,000 liters of fresh water. Any technology, such as microencapsulated diets, that might enable bivalve aquaculture to grow should be viewed as of great benefit and a worthwhile recipient of further research and industry attention.

And live algae remain a key component in the \$99.3 billion fish aquaculture industry, where application of microencapsulated diets would also be appropriate. New microencapsulated diets derived from aquacultural waste streams could contribute towards a step change in sustainable global food production through improved industry practices.

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