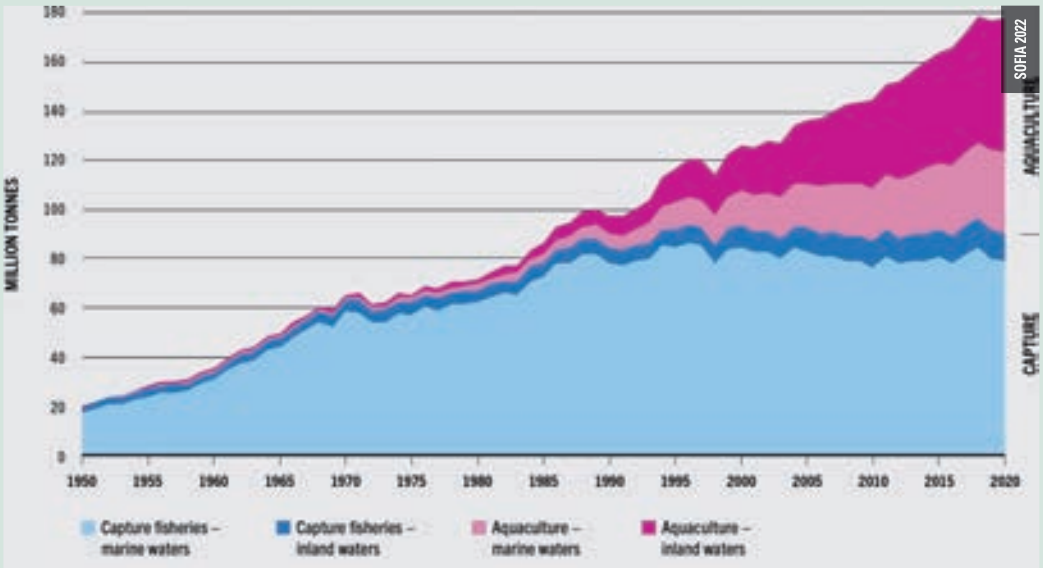
Utilising copepods as live feeds to prevent *Vibrio* spp. infections in marine fish larvae

Improved fish hatcheries could boost marine aquaculture

According to FAO's latest (2022) edition of *The State of World Fisheries and Aquaculture* marine aquaculture is growing but still produces only half the tonnage of freshwater aquaculture. When looking at fish from capture fisheries, marine species contribute by far with the largest tonnage, and there is robust demand for high value marine fishes.

One of the major bottlenecks within marine aquaculture is the supply of managed hatchery-bred fish larvae. Within freshwater aquaculture, most freshwater fish larvae are large when they emerge from the egg and can easily be fed with formulated feed pellets. For marine fish larvae, most larvae must be fed with live feeds from day one, and for some species, for up to a month after birth. Hence, in marine aquaculture hatchery production, live feeds like rotifers, *Artemia* and copepods are often the only options to produce marine fish larvae of high value species. Copepods, in particular, are important, as they are a natural live feed for fish larvae. In contrast to rotifers and *Artemia*, copepods are biochemically superior and do not need enrichment before being fed to the fish larvae. Further, copepods exhibit behaviour that triggers an attack response from many marine fish larvae.



Global capture fisheries and aquaculture production

Preventing microbial contamination of fish larvae is critical

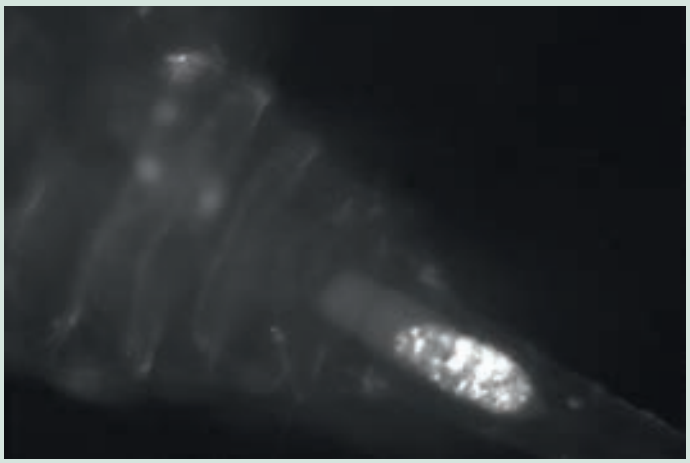
One of the obstacles to produce and use live feeds is microbial control, preventing pathogenic bacteria from being introduced to the fish larvae through the live feeds. The management of bacterial communities in live feeds has been widely studied in rotifers and *Artemia*. For example, with regard to *Artemia* research a model system has been developed to understand, control, and protect against pathogenic bacteria in *Artemia* cultures. Whereas with copepod cultivation, and its use in marine aquaculture, very few researchers are working with pathogenic bacteria control. It is an understudied but very promising area of research in marine aquaculture.

Vibrio spp. are known fish pathogenic bacteria found in both fresh- and marine aquaculture and leading to severe production and economic losses for the aquaculture sector. The *Vibrio* genera are among the most common and widespread disease-causing agents, and *Vibrio* infections play a leading role in constraining the growth of the aquaculture sector worldwide. Especially during the early larval stages of development, *Vibrio* species are a common cause of high mortality rates in reared fish. For juveniles and adult fish, vaccines are used to prevent *Vibrio*, but this is not an option in the larval stages since fish larvae are too fragile to vaccinate. Moreover, these early life stages are also where the fish larvae are highly susceptible to diseases such as *Vibrio*.

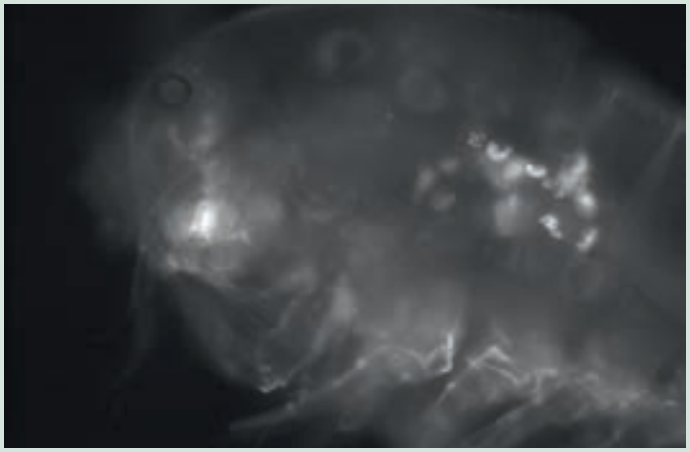
A copepod variety that produces omega-3s

At Roskilde University (Denmark) researchers have been cultivating a new promising tropical copepod species for use as live feed in marine aquaculture. The cyclopoid copepod (*Apocyclops royi*) is one of a few known copepod species that can biosynthesise fatty acids. They can be fed with, for example, yeast that only contains short-chained non-essential fatty acids and will then biosynthesise and prolong the fatty acids into long chained essential omega 3 and 6 fatty acids. Omega 3 and 6 fatty acids are just as essential in the diet for fish larvae as well as they are in the diets for humans.

Almost all other copepods need to obtain the essential fatty acids in their diets, hence the only



A faecal pellet is filled with *V. anguillarum* suggesting that the copepods ingest *V. anguillarum* together with the food into the gut system and then some is concentrated and expelled again through faeces.



A few illuminated colonies of GFP tagged *V. anguillarum* can be seen closer to the copepod's head as blurry white spots.

option is to feed them with the correct microalgae strains. This complicates copepod production and around 70% of copepod cultivation costs are solely related to the production of microalgae. So, removing this link in the production chain makes copepod cultivation easier and cheaper. Another huge advantage is that, when they are not restricted to microalgae, it is possible to experiment with other feed types that have pro- or prebiotic properties as well. Feed companies producing pelleted feeds or derivates have developed different feed products with pre- and probiotic properties, that both target potentials within the gut of the fed species, and also the surrounding aquatic cultivation medium, as a bioremediation effect.

Generating a carry over protection from copepod to larva is the goal

Now the job is "just" to find the correct feed, or the combination of feeds, and/or derivatives to try to prevent *Vibrio* infections in live feeds and secure a potential carry over effect to the marine fish larvae in hand. This "treatment" with different feed types, preventing *Vibrio* in live feed, could reveal a preventive treatment, and thus save marine aquaculture hatcheries from many losses.

A recent master's thesis from Roskilde University investigated how different feed types affected *A. royi* when infected with *Vibrio anguillarum*, a so-called challenge experiment. To ensure that *A. royi* could be cultivated on the different feed types, multiple cultures were established and fed to *A. royi* for a minimum of a month before conducting the challenge experiment. This revealed that *A. royi* could be fed on microalgae, inert feed pellets and different derivates, such as yeast. This outcome was not a surprise, since another recent study from this research group showed that *A. royi* is an ambush feeder. If it senses a prey or particle, it attacks and attempts to eat it[1]. So, a prerequisite for a feed for *A. royi* is that it needs to have the correct size and be able to stay in suspension in the culture water. After finding appropriate feed items the researchers challenged *A. royi* with different concentrations of *V. anguillarum* for three days. During this period *V. anguillarum* was tagged with a luminescent which was revealed when exposed to the correct wavelength of light (see black and white images).

Some of the other foods used in the study have, according to the producer, strong pathogen binding properties and will activate several immune receptors. Yeast derivatives are examples of foods that in some cases show strong pathogen bindings or simply physically occupy the sites inside the gut of a species where a pathogenic bacteria would normally bind.

Microalgae the most effective at preventing infection

A reduced effect of *V. anguillarium* colonies over time was observed on *A. royi* when fed some of these products. Surprisingly it was discovered that some microalgae diets were effective in preventing *V. anguillarum* infections. So, aquafeeds known to prevent *Vibrio* in shrimps did not have the same significant preventive effect in copepods, even though they are both crustaceans. On the other hand, microalgae worked very well in prevention of *Vibrio* for the three days of the experiment. This could either be due to the presence of another *Vibrio* strain or that the shrimps are a more advanced crustaceans than copepods. A possible explanation for less *V. anguillarum* infection in *A. royi* fed on microalgae could be because a diet of microalgae contains all fatty acids, and *A. royi* do not need to utilize energy to biosynthesis fatty acids, which is the case when *A. royi* are fed, for example, on yeast derivatives.

Nevertheless, interesting food candidates were found, and the next step is to carry out experiments over a longer time span. Another important step is to investigate possible carry-over effects to marine fish larvae. In other words, if a "*Vibrio* free"

1 Zempléni, A., Hansen, B. W., Kiørboe, T., & Ryderheim, F. (2022). Resolving the paradox of the ambush feeding cyclopoid copepod *Apocyclops royi* being microphagous. Journal of Plankton Research, 44(6), 936-941

copepod is fed to marine fish larvae, does one weaken or boost the fish larvae so they will have less mortality even when challenged with *Vibrio*? The team claims to have good reasons to believe that they will see enhanced effects when they feed *A. royi* with pro- or prebiotic properties to marine fish larvae.

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FIAP is a leading producer of long-lasting fishing nets

Fishing nets for commercial or recreational use

FIAP GmbH, a company in Bavaria, Germany, specialises in a wide variety of products used in the aquaculture industry. They range from devices used for water analysis, water purification, and aeration, through products related to fish processing and marketing.

One of FIAP's iconic products is the profinet Aluminum fishing net series. The company has developed fishing nets for a variety of purposes over several years. Over time, there have been many attempts to steal the designs and technologies used by FIAP, resulting in cheap products of inferior quality. However, FIAP has remained a leading producer of fishing nets on the international market due to its high quality standards and the constant improvements to its products.

FIAP profinet Aluminum—always reliable quality

The main advantage of the profinet Aluminum is that the devices are light yet sturdy due to the material they are made from. Among the features contributing to its durability and to user-friendliness is the aluminium bracket system, which is reinforced for strength and resilience. The net is pulled onto the rail in the bracket and flexibly secured with plastic beads allowing for easy removal and replacement when necessary. Moreover, the latest version features a new and improved handle clamp.

The nets themselves come in different sizes to meet every need. Options are available in terms of frame width and mesh size and spare parts such as extra nets, beads, clamps, or handles can all be ordered from the company's website. The handles can in length from 1.10 to 1.80 meters and can be chosen to fit the user's preference. Other important variables relate to the depth of the net and the width of the aluminium frame, which can range from 30 to 60 centimetres. An interesting feature of FIAP profinet Aluminum is that the depth of the net mirrors the width of the frame in each of the models. Lastly, with each size of the product, nets with different mesh sizes are available—from 5 to 20 millimetres. The nets are suited for use by commercial fish farmers, production companies, and even recreational fishermen.

The profinet Aluminum is a series of fishing nets designed for the commercial as well as the recreational user.

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