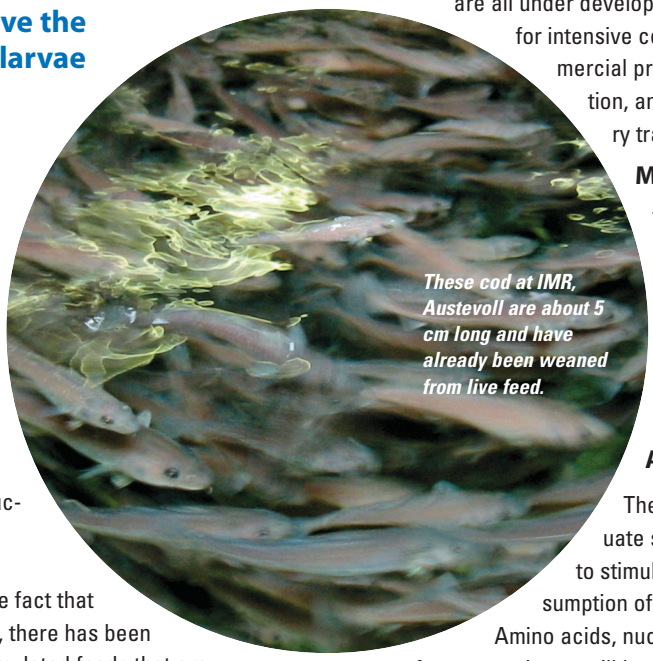
Designing improved feeds for marine fish larvae: *Norway embarks on a major program*

By Jens Kyed

- **Does smell have a significant effect on food consumption, and subsequently the ingestion-rate of fish larvae?**
- **Will adding different smells/or tastes to food simplify and improve the process of weaning fish larvae from a diet of live prey?**

A program funded by the Research Council of Norway, and conducted at the Norwegian Institute of Marine Research at Austevoll, since January 2002 has taken a new approach to these questions. A successful answer is likely to dramatically improve the rearing of marine fish larvae on formulated feeds, thus making commercial production more cost effective and efficient.



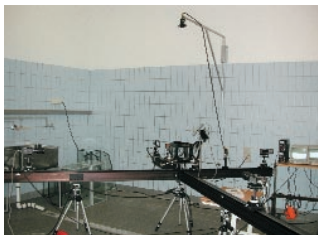
These cod at IMR, Austevoll are about 5 cm long and have already been weaned from live feed.

The research program begins with the fact that despite significant efforts world wide, there has been only limited success in producing formulated feeds that are readily ingested by fish larvae. Recent studies suggest that there could be a correlation between the food's taste and smell, and its consumption and ingestion by fish-larvae. However, the identification of odours and tastes that are favoured by larvae and which could underlie any improvement in formulated feeds, is still in its infancy. So far it has not been established what makes the difference, whether it is taste or smell or both. Dr. Howard Browman, principal research scientist at the Austevoll Aquaculture Research Station, explains. "We know that smell is an attractant for finding the food, and taste is a stimulant for ingestion. Also, it will not be only smell and taste that determines the suitability of the feed: colour, buoyancy and texture, among other factors, will also play a role."

Resources

The Norwegian Institute of Marine Research has a unique combination of facilities and expertise to conduct this work, and eight distinguished researchers are currently working on the project. The Austevoll Research Station maintains naturally spawning brood-stocks of cod, haddock, and halibut. Some fish have been photoperiod-manipulated to spawn at different times of the year, so that live larva are almost always available. Browman explains that cod, haddock and halibut have been selected because the species are all under development for intensive commercial production, and they exhibit different early life history trajectories.

All Photos by Jens Kyed



Silhouette imaging and motion analysis system

Making feed more appetising

The project is important because of the great difficulty encountered in weaning most marine fish larvae from a live diet to pelletized formulated feed. If the pellets can be made to smell and taste like real food, the fish are likely to eat more, survive better and grow more quickly.

A complicated process

The overall goal of the research is to evaluate specific substances that might be used to stimulate the feeding response and the consumption of formulated feeds by marine fish larvae. Amino acids, nucleosides, nucleotides, and extracts from natural prey will be tested in the process:

First, the researchers will try to establish, through electro-olfactogram (EOG) recordings and other electrophysiological techniques, the sensitivity of the olfactory organs to specific chemical stimuli and combinations of substances, and identify candidates for use as feeding motivators.

Second, the study aims at following the morphological-functional development of the olfactory organs and correlate this with the results from step one.

Third, the scientists will characterise the effect of these chemical stimuli on prey-searching and feeding behaviour.

Finally, using results of the first three steps, the scientists will evaluate whether one or more chemical attractants that are added to either micro-encapsulated formulated feeds or to the water, will improve the

feeding and growth rate of the larvae.

Obtaining this information is complicated and time-consuming. Of key importance are anatomical observations, electro-physiological observations in a custom built recording system, as well as behavioural studies in a specially designed instrument; the silhouette imaging and motion analysis system. The entire process must conform to the animal care regulations set down by the European Union.

In *anatomical observations* larvae are sampled for descriptions of their size and development, and for a detailed examination of the development of the olfactory organ, under light, scanning, and transmission electron microscopes.

The *Electro-olfactogram* (EOG) measures the gross responsiveness of the olfactory epithelium to olfactory stimuli. It is a multi-unit recording instrument that can be used to assess how a fish perceives an odour. EOG recordings have been obtained from cod and halibut as small as 25 mm.

The large number of tanks allows simultaneous study of different development levels of the same species.

The highly sophisticated *Silhouette imaging and motion analysis system* provides three-dimensional records of the swimming and prey-search behaviour of fish-larvae in a small aquarium. According to Browman, there are three or four of these instruments elsewhere in the world, but none is as developed as the Norwegian version, which has been interfaced with very advanced software capable of handling and classifying the images obtained.

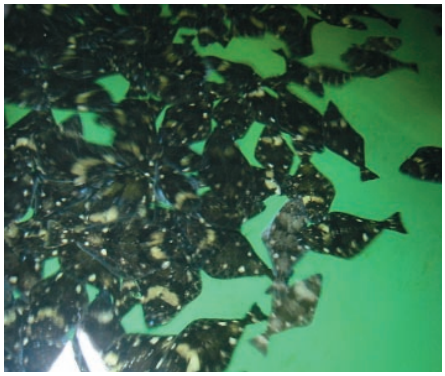
The silhouette video images allow reconstruction of larval swimming trajectories in three dimensions. The method produces a sharp shadow image of objects in the field of view. It has several advantages over standard cinegraphic or video techniques: it can be used to make detailed observations of small transparent organisms such as small fish and their prey, and events can be filmed in a large depth of field with a relatively large field of view. This allows free-swimming predators and their prey to be studied under laboratory conditions. Other features are that magnification is independent of distance from the camera, and the resolution of the system is extremely high; objects as small as 0.2 mm in diameter can be identified. Furthermore, if the test odour is mixed in seawater with a slightly different salinity than that of the water in the test tank, the odour plume will cast a shadow that is clearly visible on the recorded image. "This enables direct observation of any possible oriented movements of larvae with respect to the odour signal." said Browman.

Testing behavioural responses to odours

To observe the fish's reactions to feeds with different smells, the scientists will evaluate the behaviour of fish offered live and pelletized feeds that have been given different qualities. Some will be neutral others will have a certain smell. By studying the reactions of the larvae, the scientists will be able to correlate any increased or decreased feeding activity with a that smell. Tests will also be conducted by adding the chemical or compound directly to the water where the fish is swimming, to see if their prey search pattern changes.

The recorded images from the behavioural tests will be analysed in detail by specifically developed computer programs. Movement paths for all of the larvae are thus being generated. The movements and stationary periods, the move distances, times and velocities, turn angles, and the fractal dimension of the swim path (yielding an index of path complexity, in three dimensions), are all displayed as histograms, so that the inherent variability in the parameters is retained throughout the analysis.

Specific software is used to evaluate isolated predator-prey interactions, such as prey location and consumption. The software allows the scientists to rapidly obtain information on prey location, distances and angles. The data



(above) Young halibut. (below) Research tanks with automatic feeders.

are used to reconstruct the predator´s visual perceptual field. Taken together, the data generated by this analysis allow for a complete and detailed evaluation of the effect of added chemical compounds (smells) on the prey search pattern of larvae.

Putting the findings to the test

The results obtained from the electro-physiology and behavioural studies will be tested in growth rate experiments in order to determine whether candidate odours that were the most promising in the initial tests, if added to the water or the feed, will improve larval growth in mass culture conditions.

The current project is funded until December 2004. "However," Dr. Browman explains "as with any scientific project that yields good results, it is likely to continue - if we can obtain more funds!" Given a successful outcome, this research is likely to lead to the development of formulated feeds that are more suitable for weaning marine fish-larvae than the pelletized feeds currently used, to improve larval survival and growth rates, and lead to better cost-effectiveness.

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