Larviculture of marine finfish: the current status

In this article, the authors review the significant advances that have taken place in the larviculture of marine fish species and the main bottlenecks facing the industry.

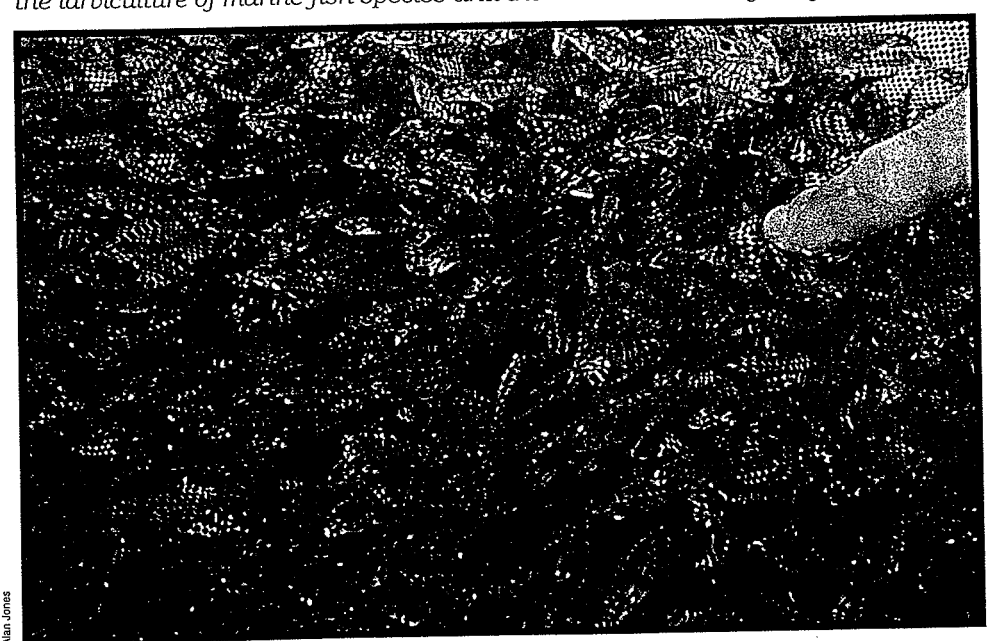
by Patrick Sorgeloos, Marleen Dehasque, Philippe Dhert and Patrick Lavens

Large scale production of marine finfish was pioneered by the Japanese with the red seabream *Pagrus major*. The Japanese Prefectural Institutes of Fisheries have been involved in the mass production of seabream since the 1960s. In Europe and the USA, research in marine fish larviculture started in the 1960s as well, albeit on a more modest scale. The expertise developed at British and French research institutes eventually led to the setting up of the first commercial hatcheries for the European seabass *Dicentrarchus labrax*, the gilthead seabream *Sparus aurata* and the turbot *Scophthalmus maximus* in the early 1980s.

Today Japan is still the biggest producer of marine fish fry with about 200 million fry produced per year. Seabream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* make up 70% of the total production, the rest consisting of puffer *Takifugu rubripes*, rockfish *Sebastes schlegeli*, mud dab *Limanda yokohamae* and several other species. Europe comes next, but with much more competitive hatchery outputs. In 1993 the fish hatcheries in the Mediterranean produced more than 100 million fry of bass and bream.

Nutritional requirements

Without any doubt, the very significant progress achieved during the last decade has been the result of improved nutrition, more particularly through the manipulation of the fatty acid profile of the live preys *Brachionus* and *Artemia*. However, the most widely used food item, the brine



Alan Jones

Juvenile seabream.

shrimp *Artemia*, of which today more than 2 000 mt of cysts are marketed annually for feeding in fish and shellfish hatcheries, has misled researchers somewhat. Because early studies revealed that the presence of the fatty acid 20:5 (n-3) (eicosapentaenoic acid or EPA) determined the suitability of a given *Artemia* source for marine fish larvae, all emphasis was on increasing the EPA levels in the live preys *Brachionus* and *Artemia* by using algae or emulsified and particulate enrichment products.

However, more attention should have been paid to the levels of 22:6 (n-3) (docosahexaenoic acid or DHA) when evaluating the results of feeding tests with different *Artemia* preparations. For the larvae of the European seabass, good survival indeed appeared to be correlated with high EPA levels in the diet; however, best growth was achieved with the diets with the highest DHA levels. Recent studies with various species have revealed the importance of DHA and, more particularly, the requirement for high DHA/EPA ratios in promoting growth, stress resistance and pigmentation. Whereas in the past DHA/EPA ratios of less than one were considered satisfactory, the emphasis now is to reach levels of 4 and higher in the live preys *Brachionus* and *Artemia* in the evaluation of diet performance. It is not unlikely that the DHA-deficient live preys that have been offered so far to marine fish larvae resulted in poor performing animals with regard to visual perception of the prey and other behavioural aspects.

The requirement in the larval diet for (n-3) highly unsaturated fatty acids (HUFAs) might also vary as a function of larval quality. This could be illustrated with turbot larvae at first feeding. Early in the turbot reproductive period, when egg and larval quality are generally at their best, the requirement for DHA enrichment in the rotifer diet is reduced to two days only, whereas late in the season, when poor quality larvae prevail, DHA needs are higher. Furthermore, a clear effect of the fatty acid composition of the maternal diet on the egg quality of *Sparus aurata* has been demonstrated. In their early larval development, turbot can turn black as a result of stressed conditions, *eg* unsuitable light conditions or elevated larval densities. Recovery to a normal colour appears to be a function of the (n-3) HUFA composition of the diet, *ie* slow when offered a coconut-enriched *Brachionus*, fast when feeding EPA and DHA enriched diets.

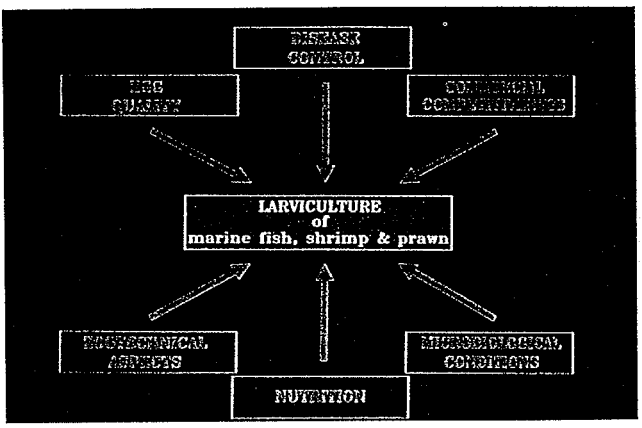
It is clear that in the field of larval nutrition the most attention so far has been paid to the qualitative and quantitive (n-3) HUFA requirements; further work is required to verify ratios of n-3/n-6/n-9 fatty acids, and especially

to evaluate the effects of other lipid classes, not least the phospholipids, which might alter the requirements for (n-3) HUFAs. Aside from the lipids it is clear that many other dietary components deserve more attention in future larviculture nutrition research, *eg* different vitamins, free amino acids, pigments, etc.

The challenge remains to analytically and biologically unravel the components present in wild copepods which are responsible for their superb dietary value for marine fish, thus eventually facilitating their incorporation in the live food chain of *Brachionus* and/or *Artemia*. Similarly, the magic effect of "green water" in the commercial rearing of most marine fish species requires further study. Various hypotheses are worth pursuing, such as its role as a source of micro-nutrients and immunostimulants, water quality conditioner, microbiological conditioner, *etc.*

Formulated diets

The ultimate goal in commercial marine fish larviculture is to be able to rely on formulated dry diets right from the start of feeding; however, for most species of marine fish this remains wishfull thinking. Nonetheless, good progress has been reported, albeit at the experimental level, with the performance of artificial diets in start-feeding of Japanese fish species and in advancing weaning in the European seabass. The application of the co-feeding principle, *ie* supplementing live food with formulated compounds, in the early larval development might provide opportunities for a better identification of qualitative/quantitative dietary requirements (*eg* with components such as phospholipids that cannot be manipulated easily in the live food fraction), for a possible reduction of live food requirements, eventually resulting in improved outputs as compared to a diet consisting solely of live food.



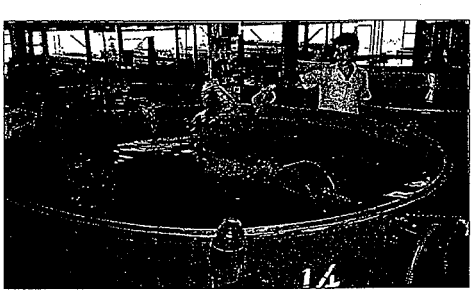
Artemia Reference Center

Scheme of interacting parameters in fish larviculture.

Microbial control

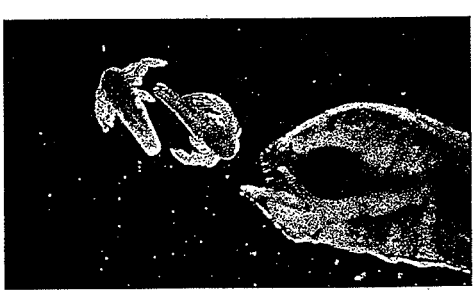
An area that urgently deserves more attention is the microbial flora of marine fish hatcheries. With the upscaling and expansion of commercial fish larviculture, hatcheries have been plagued by increased incidence of microbial diseases, often claimed to be caused by *Vibrio spp.* Similar to practices in marine shrimp hatcheries, the indiscriminate use of antibiotics in prophylactic treatment of the fish tanks resulted in the development of resistant strains and the need to switch to other antibiotics, a practice which is doomed to fail.

Thanks to support from the European Community FAR office, an extensive study has been initiated to get a better fundamental understanding of the microbial environment of two marine fish hatcheries in the Mediterranean. An extensive sampling campaign was carried out during the first 30 days of the larval rearing of seabass

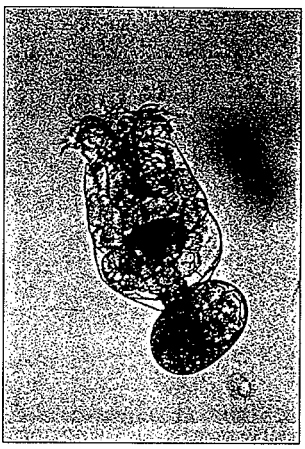


Artemia Reference Center

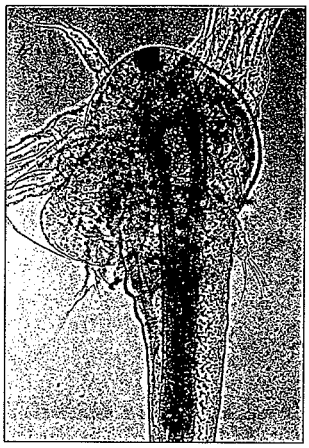
*Feeding **Artemia** to a fish larval rearing tank.*



Asian seabass larvae feeding on Artemia.

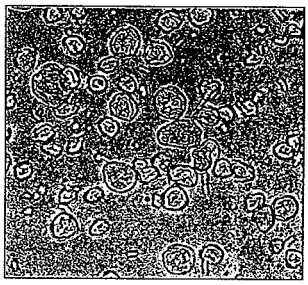


Brachionus, as seen under the microscope.



Artenia Reference Center

Enriched Artemia with oil accumulation in gut.



Artenia Reference Center

Formulated larval feeds seen under the microscope.

Dicentrarchus labrax and seabream *Sparus aurata*. The diversity and quantitative occurrence of microbes were investigated, as well as the relationship between the microflora of the culture water, the live food, and the subsequent colonisation of the larval intestine during development. The microbial diversity appears to be enormous (over 1 200 bacterial strains have been characterised) and varies from season to season as well as from hatchery to hatchery.

In one hatchery there was a clear succession of bacterial species as a function of time and live food administered; a fish mortality outbreak could be correlated with an increase in *Vibrio anguillarum* during rotifer feeding. In the other hatchery the diversity of bacterial species, including non-*Vibrios* was much higher and no larval mortalities were recorded.

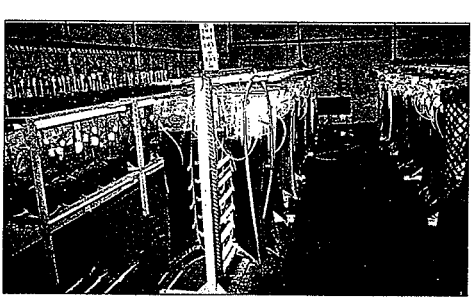
Several trials to challenge 35-40 day old seabass larvae with *V anguillarum* reference strains and isolates from the sampling campaign failed to induce mortality. It remains unclear, at least with seabass larvae, if one is dealing with obligate or opportunistic pathogens, or if the *V anguillarum* strains isolated in this study belonged to a serotype pathogenic for young larvae and not for older juveniles. In similar challenge tests with turbot *Scophthalmus maximus* larvae a study carried out by other workers could prove pathogenicity with some of the same *V anguillarum* strains.

Quantitative studies revealed the presence of 100 - 10 000 bacteria per rotifer and per brine shrimp nauplius. The bacterial numbers found in the fish larvae increase with age and type of feeding from 1 000 to 100 000 per fish larva. It is interesting to note that the numbers found on a *Vibrio*-specific TCBS-medium were always 10-100 fold lower than the total counts on a marine agar culture medium, except in cases where the composition of the microflora in the intestine of healthy fish was not an exact copy of the microflora found in the culture water or the live food. This might be an indication of selective colonisation of the gut or the existence of so-called probiotic bacteria.

The major surprise of this microbiological study was the important input of bacteria (and

Artenia Reference Center

A range of formulated larviculture feeds.



A microalgae culture room.

p. 53

potentially fish pathogens) via the live food chain. One should consider new measures to reduce bacterial loads as well as to selectively manipulate the microflora both in the live feeds produced in the hatchery and in the culture water prior to stocking of the fish larvae. Application of new disinfection procedures for *Artemia* hatching and enrichment appear to be promising. Inoculation of *Brachionus* production tanks with selected probionts results in improved rotifer culture outputs and eventually, reduced bacterial contamination in the turbot larvae. Selective bacterial inoculation of the culture tank prior to stocking with the larvae would not only reduce the chances of opportunistic bacteria becoming dominant, but eventually have a beneficial effect on first colonisation of the fish larva's gut.

In general, very strict hygienic measures should be taken in the hatcheries. Further more regular disinfection and dry-out of the complete culture circuit (including all piping) in between production cycles should be implemented. In that respect new hatcheries should consider the use of modular systems rather than to have all culture units in one building.

When antibiotic treatment is justified for therapeutic reasons, one should not add the drugs to the culture tank but rather apply oral biomedication using *Brachionus* and/or *Artemia* as a carrier for the antibiotics ingested from an emulsified or particulate preparation. Doses ranging from 20-100 ppm of sulfadrugs can be incorporated in seabass and turbot larval tissue less than 4 hours after feeding *Artemia* metanauplii boosted with antibiotics. The therapeutic efficacy of this oral delivery system has been documented with turbot larvae challenged with a *Vibrio anguillarum* pathogen.

Conclusion

The intensification of hatchery activities brought about new problems not seen on an

p.54

experimental scale, *eg* in relation to the scaling up of live food production, washing and cleaning of live food, intensity of manual labour, etc. So far too little attention has been paid to improved zootechniques which might make marine fish larviculture more predictable and more cost-effective, *eg* selection and use of new materials (such as stainless steel welded-wedge filters instead of woven filters for *Artemia* washing), increased automation so as to reduce the so-called "human factor" often responsible for reduced performances after several months of operation, etc.

In the past decade marine fish hatcheries have evolved from hit and miss ventures into profitable enterprises. In Europe, market saturation of bass, bream and even turbot fry has been reached faster than was anticipated at the World Aquaculture 1990 meeting in Halifax, Canada, *ie* fry prices have dropped to US$0.75 and less per larva.

Although there is still room for further improvements, especially with regard to cost-effectiveness, present day hatchery technology for marine fish species has proven to be widely applicable, both in terms of geographic location as well as fish species. The list of commercially cultivated species is growing fast (*e.g.* various bass and bream species, turbot, Japanese flounder, *fugu*, milkfish, mullet) and many candidate species will hopefully soon join this list, *eg* dolphin fish *mahi-mahi*, various grouper species, red snapper, halibut, cod and wolffish.

Dr Patrick Sorgeloos, Philippe Dhert and Patrick Lavens are all research scientists at the Laboratory of Aquaculture and Artemia Reference Center, University of Gent, Belgium, while Marleen Dehasque recently joined the R&D Department of INVE Aquaculture SA, Baasrode, Belgium.

The above article is an abridged version of an invited plenary lecture presented at the ICES Symposium on Mass Rearing of Juvenile Marine Fish held at the Bergen, Norway from 21-23 June 1993.

The research on which the article is based was supported by the Belgian National Science Foundation, the Belgian Ministry for Science Policy, the Belgian Administration for Development Cooperation, the European Community, SINTEF and INVE Aquaculture SA.