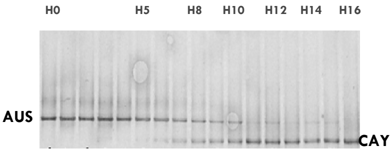
"ROTIGEN"

Shedding light on crashes in mass rotifer cultures

In aquaculture, the farming of many fish and crustacean cultures depends on the production of rotifers. Rotifers are small animals – zooplankton ranging in size from 100 to 2500 microns – that are very useful as live food for larvae because of their nutritional quality, body size and relatively slow motility. However, success in rotifer production is still one of the major bottlenecks in the further industrialization of the larviculture process. Mismanagement and bacterial contamination are parameters that cannot be neglected, while genetic reasons are getting no attention at all.



DGGE OF ROTIFER COMPOSITION IN 16 CONSECUTIVE BATCH CULTURES.
AUS: BRACHIONUS PLICATILIS 'AUSTRIA'.
CAY: BRACHIONUS PLICATILIS 'CAYMAN'.

In natural conditions, rotifer populations have an alternating asexual and sexual reproduction cycle. During the asexual reproduction (also called parthenogenesis), females produce eggs that develop without fertilization. When the environmental conditions become adverse (e.g. changes in the type of food, temperature …), males are produced and sexual reproduction takes place. This ensures a recombination of the genome, and thus an increase in the genetic diversity, as males coming from a female belonging to one clone can reproduce with a female from another clone.

The situation in commercial hatcheries is completely different. As parthenogenesis results in a faster increase in rotifer numbers and as rotifer males swim too fast for the fish larvae, farmers keep the conditions in the rotifer tanks in favour of parthenogenesis. This implies that there is little exchange of genetic material between the different clones present in the hatchery culture, which may lead to complete culture crashes. In that case, farmers exchange a relatively small number of rotifers to start a new rotifer culture, which however makes the new rotifer population suffer from a so-called "founder effect", i.e. the effect of establishing a new population by a small number of individuals, carrying only a small fraction of the original population's genetic variation. As a result, the new population may be distinctively different, both genetically and phenotypically, from the parent population from which it is derived.

In the **ROTIGEN** project, an attempt was made to shed light on the possible causes of rotifer crashes, so as to enable farmers to deal with causes rather than consequences. The following hypotheses were put forward:
1) Does the controlled mass culture of rotifers lead to an impoverishment in the genetic diversity of the cultured rotifers?
2) Does this fact in its turn make the rotifer culture more susceptible to crashes if there is a change, uncontrolled or controlled, in the biotic or abiotic conditions prevailing in the culture?

A collection of isolated rotifer clones obtained from previous research and commercial cultures was created. In order to prevent contamination of the rotifer clones by other rotifers through aerosols, rigorous protocols were established for the maintenance of this rotifer clone collection. To discriminate between the two rotifer species (*Brachionus plicatilis* and *Brachionis rotundiformis*) and the *B. plicatilis* biotypes, two genetic

Project acronym:
ROTIGEN

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Genetic implications in the production of rotifers in commercial finfish hatcheries.

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"THE SUCCESS OF ROTIFER CULTURES APPEARS TO BE AFFECTED BY THEIR GENETIC DIVERSITY AND THE RESPONSE OF THE VARIOUS ROTIFER BIOTYPES TO CHANGES IN BIOTIC AND ABIOTIC CONDITIONS"

identification techniques were used: SSCP (Single Strand Conformation Polymorphism) and DGGE (Denaturing Gradient Gel Electrophoresis). While the SSCP technique was not able to resolve all *Brachionus sp.* biotypes, the DGGE technique could easily detect all rotifer biotypes from the *B. plicatilis* species complex. Thereby, classification into one of the nine described *Brachionus plicatilis* biotypes could be achieved very quickly.

Effect of culture method (batch, semicontinuous or continuous)

This effect was tested on three *B. plicatilis s.s.* clones, 1 *B. plicatilis 'Cayman'* clone and 1 *B. plicatilis 'Nevada'* clone and considerable differences were detected among rotifer clones. The *Nannochloropsis oculata* paste supported a good growth of all tested rotifer biotypes, while feeding *Culture Selco 3000* resulted in low growth of *B.plicatilis 'Nevada'*.

Effect of stable versus variable (a)biotic conditions

An experiment with four feeding regimes, based on *Nannochloropsis oculata* and *Culture Selco 3000* was conducted. Feeding a rotifer culture with fluctuating diets, alternating algae or *CS-3000*, sustained longer a diverse rotifer population than feeding solely one of the 2 diets.

Effect of microbial stability

A technique using glutaraldehyd was developed to obtain uncontaminated rotifers, which enabled to do research on the effect of the following micro-organisms: *Phenylobacterium sp.*, *Gluconobacter sp.* and *Paracoccus denitrificans*. The density of *Phenylobacterium sp.* had no effect on *B. plicatilis s.s.*, but had negative effects on *B. p. 'Nevada'*. A density increase of this bacterium resulted in a lower rotifer density, while the density of *B. p. 'Cayman'* increased. Whether these bacteria act as additional feed or deliver essential spore elements remains to be investigated.

The success of rotifer cultures thus appears to be affected by their genetic diversity and the response of the various rotifer biotypes to changes in biotic and abiotic conditions. As concerns the impoverishment of genetic diversity in mass rotifer cultures, analysis of the hatchery samples showed that the hatchery cultures tended to start from samples with minimal or no species variation, or, if that was not the case, other culture conditions (pH, feeding, etc.) lead to the dominance of a single biotype. This poor genetic diversity might contribute to the occurrence of rotifer crashes in hatcheries, although most probably it is a multi-factorial problem in which people may play a significant role.