Gnotobiotic models for seabass (*Dicentrarchus labrax* L.) and Dover sole (*Solea solea* L.): the chain is only as strong as its weakest link...

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Despite the fast expansion of aquaculture in the last decennia, major losses during larval production still torment the industry. These losses are mainly caused by bacterial diseases, and combating these diseases by means of antimicrobial agents causes an increase in acquired antimicrobial resistance. The use of probiotics is a promising alternative treatment technique, although their working mechanism is still poorly understood. To unravel their mode of action, there is a great need for a gnotobiotic model.

The advantage of working with a gnotobiotic model is that the microbial community is known, eliminating the interference by unknown microflora.

Seabass (*Dicentrarchus labrax*) and Dover sole (*Solea solea*) are both important species for the European aquaculture industry, of which the larval culture poses a major challenge hence the justification for the creation of a gnotobiotic model. For seabass, a gnotobiotic model was developed by Dierckens et al. (2009). Regarding sole, a gnotobiotic model currently is non-existing. Pinpointing/developing a gnotobiotic model for both species, without having to house the larvae in antibiotics, is a major challenge. During the development of such a model, many different pitfalls can be encountered, as listed below.

1. All used material should be sterile and all manipulations should be carried out under strict sterile conditions

   - Use of sterile gloves, autoclaved material, autoclaved seawater
   - Sterilisation procedure and manipulations in a laminar flow

2. Finding the sterilisation protocol with a good balance between axenity and hatchability of the eggs

   **Seabass**
   - Many different protocols and products are tested:
     - H2O2, ozone, glutaraldehyde, antibiotic mixtures, plasma sterilisation, ...
   - Combinations of these products
   - Best working protocol:
     - 1% H2O2 (5 min) + ozone (3 min)
     - Overall: good tolerance to different disinfectants, good hatchability

   **Sole**
   - Most promising protocol:
     - 1% H2O2 (3 min) + 400 ppm glutaraldehyde (2.5 min) + antibiotic mixture (rinsed before hatching)
     - Overall: low tolerance to disinfectants, lower hatchability

   Why the difference in tolerance?

   Ultrastructure of the egg

3. Evaluating the axenity of the retrieved egg/larva in a quick and reliable way

   - **Culture dependent techniques:**
     - widely used
     - selective media and need for a long incubation period
   - **Culture independent techniques:**
     - also non-culturable bacteria, quick method
     - difficult to interpret

   Culture dependent techniques: TCBS, MA, TSB+ 2% NaCl

   Control (bacteria: arrow) vs most promising treatment

   Culture independent techniques: Flow cytometry

4. Maintaining axenity of the larvae during development

   - Larvae are housed in well plates
   - Wells are placed in a glove box
   - Food (Artemia) has to be axenic

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