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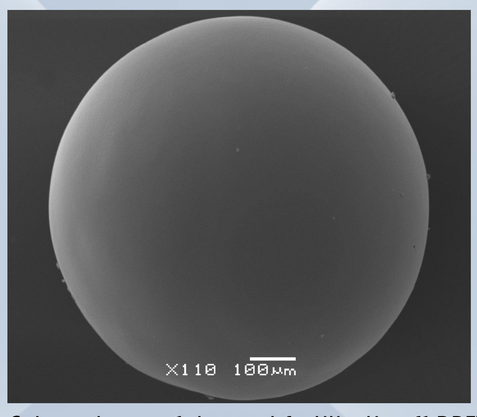
Evelien De Swaef

ELECTRON MICROSCOPIC CHARACTERIZATION OF FERTILISED DOVER SOLE *SOLEA SOLEA* EGGS DURING EMBRYONIC DEVELOPMENT

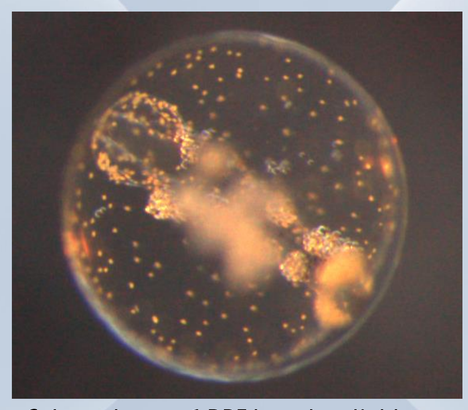
De Swaef Evelien[1], Claeys Myriam[2], Bert Wim[2], Huysseune Ann[3], Witten Paul Eckhard[3], Van den Broeck Wim[1], Decostere Annemie[4]
[1]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[2]Nematology Research Unit, Department of Biology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium
[3]Evolutionary Developmental Biology, Department of Biology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium
[4]Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Introduction



Solea solea egg 1 day post fertilisation (1 DPF) based on scanning electron microscopy (SEM)

Dover sole (*Solea solea*) is a very promising aquaculture candidate for European aquaculture due to its high flesh quality and market value. Until now, **no data** on the ultrastructural morphology of the egg envelope is available although this information could increase the knowledge on the development of Dover sole eggs and facilitate larviculture practices. Furthermore, the ultrastructural morphology of the teleost fish egg is frequently used for species identification and phylogenetic classification. Considering the above, the aim of this study is **to investigate the overall ultrastructural morphology of the egg envelope of Dover sole embryos during development.**



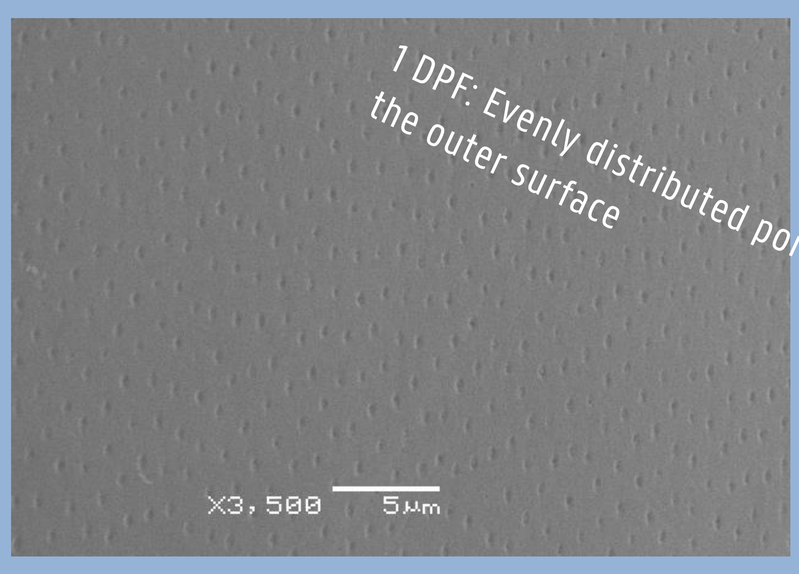
Solea solea egg 1 DPF based on light microscopy

Methods

In first instance, **>35 fixation and embedding protocols** were assessed. The successful protocol for **scanning electron microscopy** (SEM) combined fixation with 4% glutaraldehyde in 0.1 M cacodylate buffer for minimum 4 h with post-fixation of 2 h with 1% OsO4 in 0.1 M cacodylate buffer. For **transmission electron microscopy** (TEM), **puncturing** the egg envelope during the first steps of the fixation protocol was necessary to allow the embedding medium to penetrate through the egg envelope. Secondly, SEM and TEM examination took place.

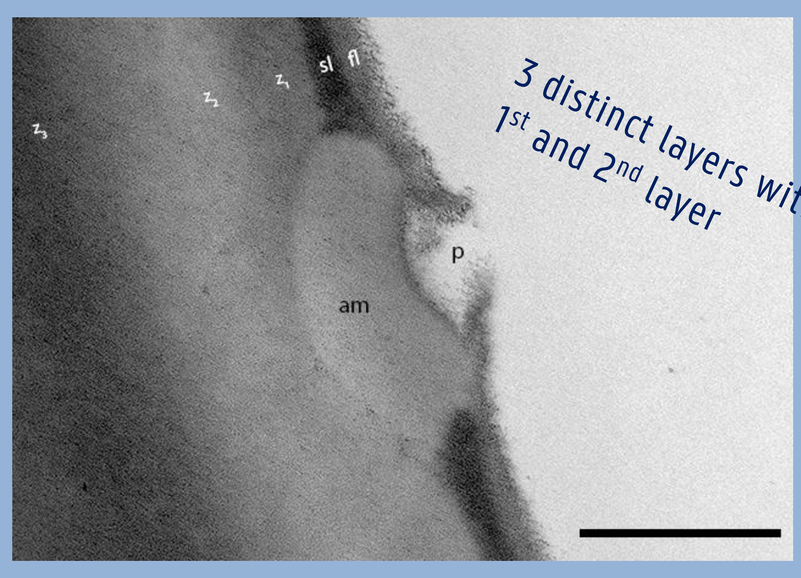
Results

Scanning electron microscopy



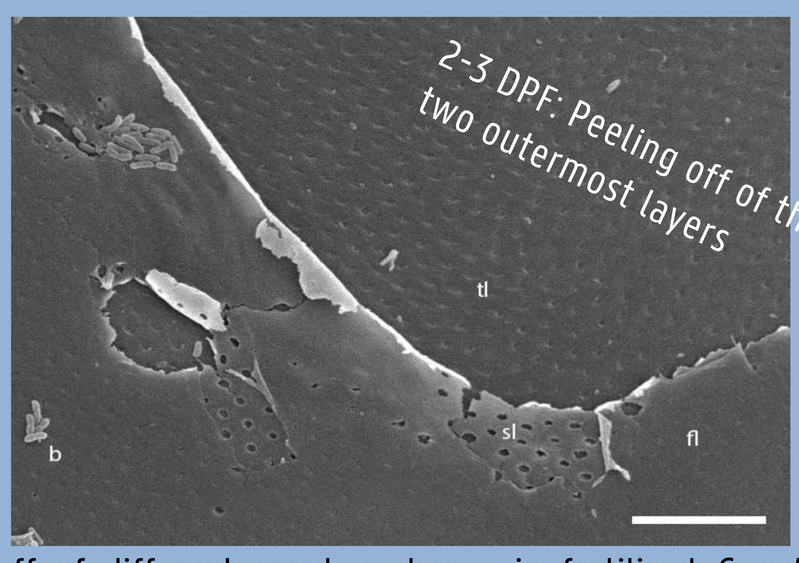
Evenly distributed pores on the outer surface in fertilised *S. solea* eggs sampled at 1 day post fertilisation (DPF). Bar = 5µm.

Transmission electron microscopy

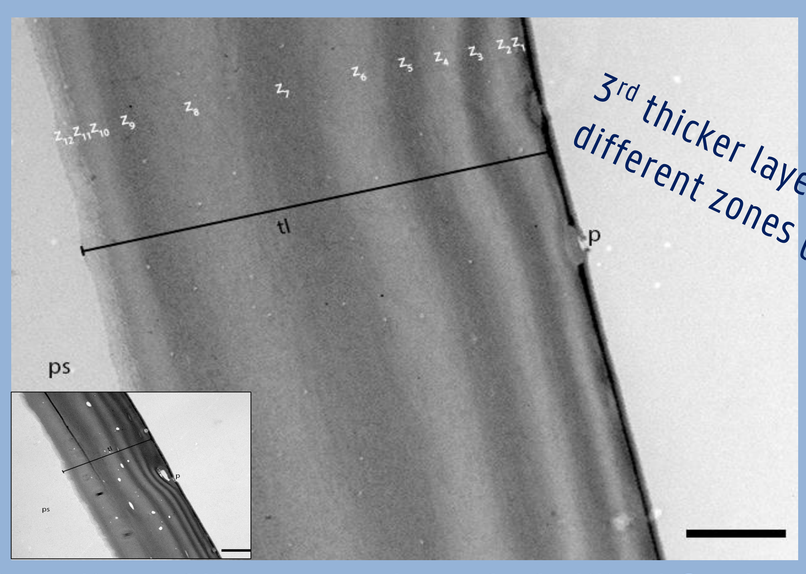


Detail of a pore in the external layers of the egg envelope of a fertilized *S. solea* egg (1 DPF). fl: first outermost layer of the egg envelope, sl: second deeper layer, z1-z3: zones of alternating electrondensity of the third innermost layer, am: amorphous material, p: pore. Bar = 500 nm.

Not confirmed by TEM images



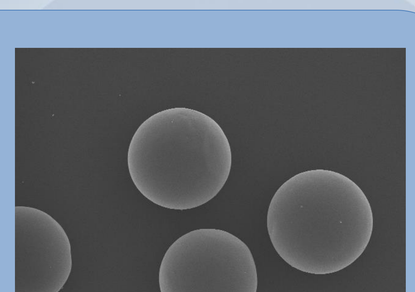
Peeling off of different envelope layers in fertilised *S. solea* eggs sampled at 2 DPF. fl: first outermost layer of the egg envelope, sl: second deeper layer of the egg envelope, tl: third innermost layer of the egg envelope. Bar = 10 µm.



Ultrathin sections of the egg envelope of a fertilised *S. solea* egg (1 DPF), showing three distinct layers.. tl: third innermost layer of the egg envelope consisting of 12 zones of alternating electrondensity (Z1 till Z12), ps: perivitelline space, p: pore. Bar = 2 µm.
Insert: Holes and tears are visible in the third innermost layer at 4 DPH.

Take home message

Based on **both SEM and TEM examination**, **three distinct layers** were determined in the egg envelope. Following **SEM examination**, **peeling off of the two outer layers** during embryonic development could be noticed, revealing a **change** in the morphology of the pores evenly distributed on the envelope surface. The three distinct layers of the envelope were also observed based on **TEM**, additionally accentuating the compact structure of the **innermost layer** which was distorted by **dispersed holes and tears** close to hatching.



Solea solea eggs 1 DPF (SEM)

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Evelien.DeSwaef@ugent.be