pseudo-4D visualization of a flatfish head during metamorphosis

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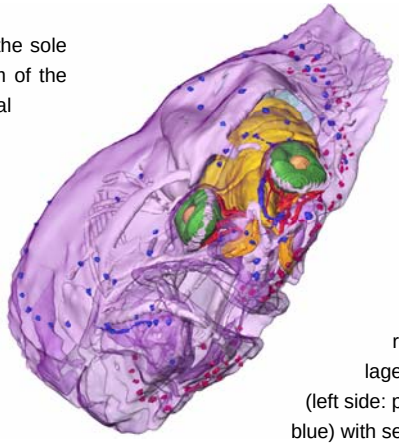
Selected histological transversal sections through a larval stage of Solea solea - migrating eye at dorsal position - from snout (left) to otic capsule with labyrinth organ (right).

Introduction

The developing flatfish head undergoes striking morphological changes from hatching through metamorphosis: coming along with eye migration some organ systems grow asymmetrically, some retain their original bilateral symmetry. Questions arise about the presence of solid obstacles on the path of the migrating eye, or about geometrical changes in the vestibulo-oculomotoric system, etc. Whereas several details of this complex process are well known, up to now no attempt was undertaken to show all organ systems involved together to elucidate their stereological interdependencies.

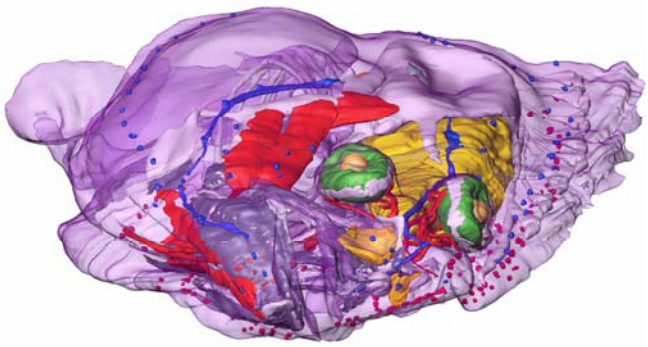
Material & Methods

Serial semithin sections through the heads of three larval stages of the sole (*Solea solea*) were made from the snout-tip to the foramen magnum of the otic capsule (altogether 2770 slices). Digital images of each mechanical slice were made on a light microscope, imported into the 3D-rendering software Amira, converted to stacks, aligned and segmented manually. Then the abstracted organ contours were used to compute surface-models of several organ systems, to display these "materials" at any angle of view and to carry out some procedures of morphometric analysis.



Results

Growth and differentiation of the larval head of *Solea solea* with most of its organ systems is illustrated at 1, 3 and 4 weeks post-hatching (from top-right to bottom-left): integument (violet), brain and optic nerves (yellow), olfactory epithelia and lenses (orange), retinae (green), jaw- and eye-muscles (red), cartilage (light grey), bone (dark grey), lateral line system (left side: purple, right side: dark blue), labyrinth organ (light blue) with sensory epithelia (pink). During translocation of the left eye to the right (upper) side in *S. solea* the left nervus opticus gets slightly elongated but no solid obstacle of the chondrocranium or osteocranium can be found on the migrating eye's way: e.g. on both sides the taeniae marginales of the otic capsule remain short and do not contact the laminae orbitonasales (to form supraorbital cartilages like in the turbot). Details get visible, e.g. a deflection of the nervus opticus at the decussations with the musculus rectus nasalis in symmetric larval stages, later a slight enlargement of the right crista horizontalis and a deformation of the left canalis supraorbitalis of the lateral line system appear …



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Discussion

The 4D-data set created in this study provides the possibility to display single or several organ systems of the sole head in any combination, transparency and angle of view at different developmental stages – sort of handling a virtual "transparent fish" in space and time. This allows detailed insight in the complex morphogenesis of the flatfish head during metamorphosis, e.g. the effects of eye displacement to all neighbouring tissue structures involved. Furthermore torsions and left-right-asymmetries can be visualized and quantified, concerning especially the anterior parts of brain, cranium and lateral line system, the olfactory organs and the jaw apparatus. However, the bulk of the brain and cranium and the labyrinth organ with its sensory epithelia remain unaltered in their original symmetry to a far extend. Currently the same methods are applied to the larvae of the turbot *Psetta maxima*.

Selected reconstructions of the Solea head seen from frontal-right: cartilage + bones, cartilage + muscles, side channel system, eyes + muscles, brain + nostrils, labyrinth system.