ESRF	**Experiment title:** Ontogeny of the extant coelacanth *Latimeria chalumnae*	**Experiment number**: EC-1023
Beamline: ID19	**Date of experiment**: from: 14/09/2012 to: 17/09/2012	**Date of report**: 01/03/2020
Shifts: 6	**Local contact(s)**: Paul Tafforeau	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Hugo Dutel*

Report summary:

The coelacanth *Latimeria* is an iconic fish closely related to land dwelling vertebrates, the tetrapods. *Latimeria* is critical for our understanding of the origin of tetrapods, but its development has remained virtually unknown since its discovery in 1938. To fill this gap, we gathered specimens of different developmental stages housed in natural history collections, and performed high resolution phase contrast X-ray synchrotron microtomography on this unique developmental series. The sum of data collected at the ESRF allowed us to study the development of the most remarkable anatomical features of *Latimeria*, such as its hinged braincase and brain (Fig. 1). This work represents signficant contributions to our understanding of vertebrate evolution. These studies have been published in top peer-reviewed journals, and attracted media coverage in France and abroad. Raw data obtained at the ESRF and 3D digital reconstructions associated with publications were made publicly available on online repositories (http://phenome10k.org and http://paleo.esrf.fr).

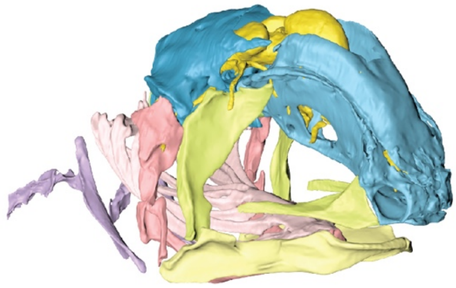

Figure 1 3D digital reconstruction of the head structures of a coelacanth embryo (Dutel et al. 2019 *Nature*). The braincase is colored in blue, and houses the brain (yellow).

Peer-reviewed publications:

Dutel H., Galland M., Tafforeau P., Long J.A., Fagan M.J., Janvier P., Herrel A., Santin M.D., Clément G., Herbin M. (2019) *Nature* **569**: 556-559. https://doi.org/10.1038/s41586-019-1117-3

Abstract: The neurocranium of sarcopterygian fishes was originally divided into an anterior (ethmosphenoid) and posterior (otoccipital) portion by an intracranial joint, and underwent major changes in its overall geometry before fusing into a single unit in lungfishes and early tetrapods. Although the pattern of these changes is well-documented, the developmental mechanisms that underpin variation in the form of the neurocranium and its associated soft tissues during the evolution of sarcopterygian fishes remain poorly understood. The coelacanth *Latimeria* is the only known living vertebrate that retains an intracranial joint. Despite its importance for understanding neurocranial evolution, the development of the neurocranium of this ovoviviparous fish remains unknown. Here we investigate the ontogeny of the neurocranium and brain in *Latimeria chalumnae* using conventional and synchrotron X-ray micro-computed tomography as well as magnetic resonance imaging, performed on an extensive growth series for this species. We describe the neurocranium at the earliest developmental stage known for *Latimeria*, as well as the major changes that the neurocranium undergoes during ontogeny. Changes in the neurocranium are associated with an extreme reduction in the relative size of the brain

along with an enlargement of the notochord. The development of the notochord appears to have a major effect on the surrounding cranial components, and might underpin the formation of the intracranial joint. Our results shed light on the interplay between the neurocranium and its adjacent soft tissues during development in *Latimeria*, and provide insights into the developmental mechanisms that are likely to have underpinned the evolution of neurocranial diversity in sarcopterygian fishes.

Cupello C., Brito P. M., Herbin M., Meunier F., Janvier P., Dutel H., Clément G. (2015) Allometric growth in the extant coelacanth lung during ontogeny. *Nature Communications* **6**: 8222.
https://doi.org/10.1038/ncomms9222

Abstract: Coelacanths are lobe-finned fishes known from the Devonian to Recent that were long considered extinct, until the discovery of two living species in deep marine waters of the Mozambique Channel and Sulawesi. Despite extensive studies, the pulmonary system of extant coelacanths has not been fully investigated. Here we confirm the presence of a lung and discuss its allometric growth in *Latimeria chalumnae*, based on a unique ontogenetic series. Our results demonstrate the presence of a potentially functional, well-developed lung in the earliest known coelacanth embryo, and its arrested growth at later ontogenetic stages, when the lung is clearly vestigial. The parallel development of a fatty organ for buoyancy control suggests a unique adaptation to deep-water environments. Furthermore, we provide the first evidence for the presence of small, hard, flexible plates around the lung in *L. chalumnae*, and consider them homologous to the plates of the 'calcified lung' of fossil coelacanths.

Mansuit R., Clément G., Herrel A., Dutel H., Tafforeau P., Santin M.D., Herbin M. (2020) *Journal of Anatomy* **236**:493-509. https://doi.org/10.1111/joa.13115

Abstract: The monobasal pectoral fins of living coelacanths and lungfishes are homologous to the forelimbs of tetrapods and are thus critical to investigate the origin thereof. However, it remains unclear whether the similarity in the asymmetrical endoskeletal arrangement of the pectoral fins of coelacanths reflects the evolution of the pectoral appendages in sarcopterygians. Here, we describe for the first time the development of the pectoral fin and shoulder girdle in the extant coelacanth *Latimeria chalumnae*, based on the tomographic acquisition of a growth series. The pectoral girdle and pectoral fin endoskeleton are formed early in development with a radially outward growth of the endoskeletal elements. The visualization of the pectoral girdle during development shows a reorientation of the girdle between the fetus and pup 1 stages, creating a contact between the scapulocoracoids and the clavicles in the ventro-medial region. Moreover, we observed a splitting of the pre- and post-axial cartilaginous plates in respectively pre-axial radials and accessory elements on one hand, and in post-axial accessory elements on the other hand. However, the mechanisms involved in the splitting of the cartilaginous plates appear different from those involved in the formation of radials in actinopterygians. Our results show a proportional reduction of the proximal pre-axial radial of the fin, rendering the external morphology of the fin more lobe-shaped, and a spatial reorganization of elements resulting from the fragmentation of the two cartilaginous plates. *Latimeria* development hence supports previous interpretations of the asymmetrical pectoral fin skeleton as being plesiomorphic for coelacanths and sarcopterygians.