EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Ultrastructure and mechanical behavior of calcified shark cartilage	**Experiment number**: EC429
Beamline:	**Date of experiment**: from: 15 Jul 2009 to: 17 Jul 2009	**Date of report**: 1 March 2011
Shifts:	**Local contact(s)**: TAFFOREAU, Paul	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Mason N. Dean*, University of California Irvine, USA; Proposer

Non-proposer experimentalists:

Dominique Adriaens*, University of Ghent, Belgium

Kerin Claeson*, University of Texas Austin, USA

Emilie Descamps*, University of Ghent, Belgium

Report:

The primary goal of our proposal "EC-429: Ultrastructure and mechanical behavior of calcified shark cartilage" was to generate high-resolution tomographic data for the curious "tiled" calcified cartilage from several species of elasmobranch fish (sharks, rays and relatives). This morphologically distinct tissue is, phylogenetically, the oldest extant example of vertebrate mineralized tissue and so can provide deep insight into mineralization pathways and the evolution of skeletons. We had previously gathered tomographic data from a single species at the Argonne National Lab, but found the resolution of that beam source to be inadequate for the ultrastructural features we were discovering. Our beamtime allowed us to scan tissue from a huge taxonomic diversity, encompassing 2-3 different skeletal elements (jaws, hyomandibulae, crania) from 12 species of batoids (rays, skates and relatives) and 4 species of sharks. It also allowed us to test the efficacy of a new freeze-drying sample preparation technique – our previous samples from the ANL had been scanned wet, often resulting in unfixable "settling"/movement artifacts. Our ESRF beamtime illustrated the effectiveness of the freeze-drying method, which allowed easier specimen transport and resulted in highly detailed scans with few/no artifacts.

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Our beamtime resulted in a massive amount of data, which we are still analyzing. I have since begun a post doc at the Max Planck Institute of Colloids & Interfaces where I am working with a student to refine the scan reconstructions and write code to perform automated morphological analysis of the calcified tissue. Our work at the ANL and ESRF led us to the discovery of small canals within the calcified matrix, which apparently allow chondrocytes (cartilage cells), entombed in mineralized cell spaces, to remain alive. With the higher resolution ESRF data, we are working to map the canalicular networks and quantify spatial variation in the dimensions of the canals and cell spaces; these data can then not only act as tests of our hypotheses of how the mineralized tissue grows (e.g. we expect smaller canals/cell spaces deeper within the mineralized tissue, indicating that the deeper cells gradually "wall themselves in" with mineral), but also describe the phylogenetic variation in the tissue's micro-morphology, which has never been attempted due to technological constraints. We are working with our student researcher to complete this project by the fall of 2011.

We are also working to extend this work into a smaller scale of structural hierarchy through diffraction analyses of the crystal structure and organization of shark calcified cartilage. Paired with our previous tomographic work, this would allow a profound understanding of the tissue's organization over a broad size range. We have performed preliminary scattering experiments at the BESSY II µ-Spot Beamline and hope to expand these studies with additional beamtime at the ESRF ID01.

In addition to our cartilage samples, we were able to perform additional methodological tests as part of two ongoing projects in Dominique Adriaens lab at the University of Ghent on (1) craniofacial development in *Xenopus* frogs, and (2) musculoskeletal anatomy of the prehensile tail in seahorses. Specimens of tadpoles and newborn seahorses were postfixed with osmiumtetroxide, in order to contrast soft tissues, then scanned to evaluate to what degree useful information could be obtained using synchrotron scanning. These first trials showed that specimen preparation will need to be improved before proper results can be obtained, as (1) tadpole specimens experienced a substantial deformation during scanning, and (2) the miniature seahorses were unstable during scanning. However, one trial on a juvenile seahorse did yield a scan that allows discrimination between skeletal and some soft tissue components, and is currently incorporated in a comparative study of the caudal musculoskeletal system in syngnathid species (seahorses, pipefishes and relatives).