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 via the User Portal:

*https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do*

***Reports supporting requests for additional beam time***

Reports can 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:**<br>"Rocking earstones: In-situ investigation of otolith motion in the ear of bony fishes" | **Experiment number:**<br>LS-2539 |
|---|---|---|
| **Beamline:**<br>ID17 | **Date of experiment:**<br>from: 24 September 2016 to: 26 September 2016<br>from: 22 February 2017 to: 23 February 2017 | **Date of report:**<br>22.05.2017 |
| **Shifts:** 9 | **Local contact(s):**<br>Dr. Alberto Mittone, Dr. Alberto Bravin | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

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## Report:

Aim: First detailed experimental analysis of fish otolith motion including in-situ measurements in fresh-dead animals.

Specific Research Questions: see report LS-2539 – ID19

Sound stimulus (2. session): "no sound period (2 sec) – sound period (ν = 0.2 kHz, pure tone, 5 sec) – no sound period (2 sec)" presented in 3 consecutive repeats at a sound pressure level (SPL) of 156 dB re 1 µPa (SPL - ambient noise, no stimulus presentation: 107 dB re 1 µPa). The ambient noise spectrum revealed high relative amplitudes in the low frequency range at about 0.3 and 0.55 kHz (Fig. 1A). As the SPL of 107 dB was distinctly lower than that measured when presenting the 0.2 kHz stimulus, this is unlikely to impede the experiments. More importantly, as seen at ID19, no otolith motion was detected during the no sound periods.

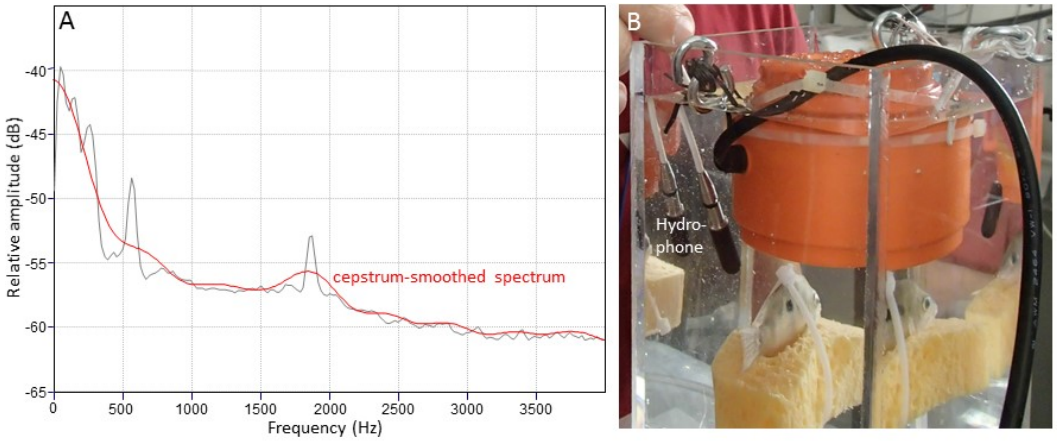


**Fig. 1.** Ambient noise spectrum (A) measured in the rectangular test tank during the 2. session in February 2017 using a Brüel & Kjær hydrophone 8103 (B).

Test subjects:

(1) Simplified "fish ear model": Set-up for investigating the motion of isolated otoliths.
Otoliths embedded in 0.5% agarose:
*Steatocranus tinanti* (*N*=1)
*Etroplus maculatus* (*N*=2)

(2) In-situ system, i.e. fresh-dead animals (Fig. 1B):

*Steatocranus casuarius* (*N*=3), *S. tinanti* (*N*=1): no swimbladder-ear connection; "hearing generalist"
*Etroplus maculatus* (*N*=6): swimbladder-ear connection present; "hearing specialist"

Set-up Issues & Set-up Optimization:

*Set-ups in September 2016*

We used a large cylindric tank (inner diameter: 22 cm; volume: 9.5 l) with (1) an underwater speaker (UW-30, max. diameter 18.3 cm) suspended from above or (2) an air loudspeaker (Monacor SPH 170c) fixed closely above the water surface. The large water body caused considerable attenuation of the beam. Moreover, the curved wall of the cylindric tank had also a negative effect on the achievable resolution and SNR. However, from an acoustic viewpoint the larger tank would have been preferable due its better acoustic properties (less issues with sound reflection).

*Tomography vs. 2D radiography*

As tomography during sound presentation was not successful, i.e. the scan was interrupted, we tested the effect of sound presentation at 0.2 and 0.5 kHz with 2D radiography in xyt (lateral), xzt (dorsal), and yzt (frontal) views (1) in the large cylindric tank using the UW-30 or the air loudspeaker or (2) a smaller rectangular tank (inner dimensions: 9 cm x 9 cm x 15 cm) using the air loudspeaker.

*Air Loudspeaker vs. Underwater speaker (UW-30)*

The air loudspeaker could not transfer enough sound pressure into the water to set the fish and its otoliths into motion. Thus, we could only proceed with the underwater speaker and the large cylindric tank.

Outcomes of the 1. session (September 2016):

It turned out that only a smaller rectangular tank in combination with a smaller underwater speaker was suitable to improve the signal-to-noise ratio while producing sufficient sound pressure; although the tank acoustics are likely to be worse than in the large cylinder. As the maximum framerate was fixed to 124.98 fps, it was not possible to directly detect otolith motion in-situ. Thus, for the 2. session, we decided to use a simplified "fish ear model" to test which settings may provide a successful visualization of otolith motion in-situ.

Improved Set-up (2. session, February 2017):

We used a rectangular tank (inner dimensions: 10 cm x 10 cm x 20 cm) with a small underwater speaker (Daravoc MA001, max. diameter 6.7 cm) (Fig. 2A). In this set-up, we obtained a much improved signal-to-noise ratio. In addition to whole fresh-dead fishes, we used single otoliths embedded in 0.5% agarose as a simplified model (Fig. 2B).

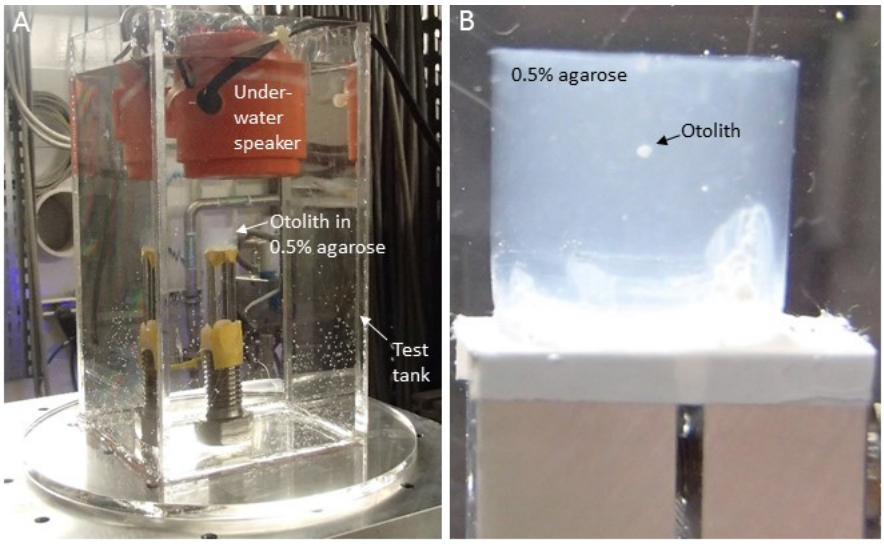


**Fig. 2.** Improved set-up using the rectangular tank and the small underwater speaker (A). (B) shows the simplified "fish ear model".

Outcomes & Conclusions:

In the simplified "fish ear model", slight movements of the otolith were discernable; however, the non-adjustable framerate of max. 124.98 fps remained a main issue. This could be successfully fixed at ID19 for the 0.2 kHz sound stimulus (see our separate report for ID19). It has to be emphasized that our experiments at ID17 have been indispensable to optimize the overall set-up that very successfully worked at ID19.

| ESRF | **Experiment title:** "Rocking earstones: In-situ investigation of otolith motion in the ear of bony fishes" | **Experiment number**: LS-2539 |
|---|---|---|
| **Beamline**: ID19 | **Date of experiment**: from: 23 February 2017 to: 24 February 2017 | **Date of report**: 22.05.2017 |
| **Shifts:** 3 | **Local contact(s)**: Dr. Alexander Rack, Dr. Margie Olbinado | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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## Report:

Aim: *First detailed experimental analysis of fish otolith motion including in-situ measurements in fresh-dead animals.* – For the first time, otolith motion could be successfully visualized in-situ.

Specific Research Questions & Preliminary Results:

(1) *Does otolith motion induced by a 0.2 or 0.5 kHz sound stimulus differ within the otolith (e.g. margin vs. center)?*

For the studied 0.2 kHz stimulus, the otoliths embedded in agarose seem to move as a "rigid" body.

(2) *How does otolith motion in one species differ between different sound stimuli (0.2 vs. 0.5 kHz) and between different otoliths (3 otolith pairs per individual)?*

As the 0.5 kHz stimulus could not be successfully tested – sufficient adjustment of a framerate close to 500 fps with evaluable signal-to-noise-ratio was not possible due to the heavily absorbing water body in the test tank – the first part of the question could not be answered yet. The different otolith types (lapilli vs. sagittae and asterisci) seem to show different motion patterns as expected by their different in-situ orientation and amount of attachment to the respective sensory epithelium which they overlie.

(3) *How does otolith motion differ between species at the same stimulus frequency and between stimuli, respectively?*

Yet, sample sizes are not large enough to evaluate whether there is a species-specific difference or not.

Sound stimulus: "no sound period (2 sec) – sound period (ν = 0.2 kHz, sine tone, 5 sec) – no sound period (2 sec)" presented in 3 consecutive repeats at a sound pressure level (SPL) of 157 dB re 1 µPa (SPL - ambient noise, no stimulus presentation: 115 dB re 1 µPa).

Test subjects:

(1) Simplified "fish ear model": Otoliths embedded in agarose

Test if a characterization of otolith motion in a sound field is possible by using hard X-rays (phase contrast imaging) while adjusting the framerate.

*S. tinanti* (*N*=1): 1 otolith in 1% agarose

*E. maculatus* (*N*=2): 1 otolith in 0.5% agarose, 1 otolith in 1% agarose

(2) In-situ system, i.e. fresh-dead animals:

As the simple model provided good results (Fig. 1A), we studied otolith motion in-situ in dorsal and lateral views.

*Steatocranus tinanti* (*N*=1): no swimbladder-ear connection; "hearing generalist"

*Etroplus maculatus* (*N*=2): swimbladder-ear connection present; "hearing specialist"

Visualization of otolith motion:

(1) Measurement of the simple "fish ear model" (Figs. 1, 2A):

Embedding in 1% agarose was preferred over 0.5% agarose, as in the latter numerous bubbles quickly emerged during the scanning procedure. The adjusted framerate of 198.020 fps with respect to a 0.2 kHz sound stimulus allowed to visualize the otolith motion in lateral and dorsal views with the predicted beat frequency of 2 Hz (Fig. 1B). It could be further shown that sound impinging mainly from above not only provokes motion along the vertical axis but at the same time leads to otolith motion along the horizontal axis.

(2) Measurement of otoliths in-situ:

Different motion patterns of lapilli vs. sagittae/asterisci: In dorsal view (Fig. 2), sagittae and asterisci moved in both views (dorsal and lateral) mainly along the vertical (sound) axis, while lapilli also displayed distinct movement along the horizontal axis.

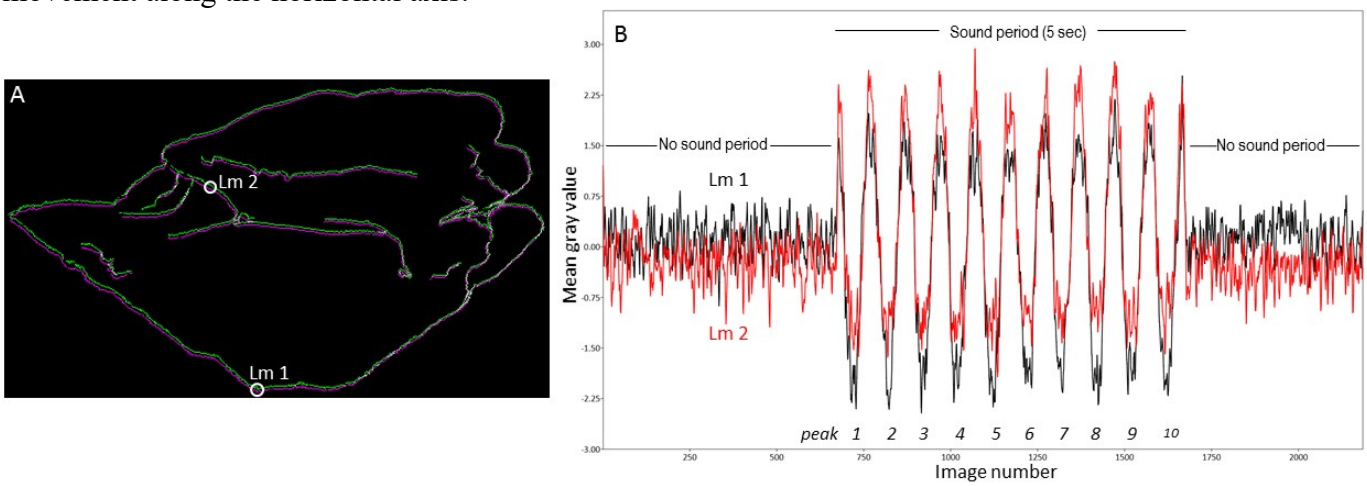


**Fig. 1.** The motion at 2 landmarks (Lm) of the *E. maculatus* sagitta is illustrated by the overlay of the averaged maxima (green) and minima (purple) outlines (A) and was quantified in ImageJ (1.51n) applying the Plot-Z-Profile procedure (B). Both Lms clearly show the predicted beat frequency of 2 Hz. The vertical amplitude is ca. 20 µm under current set-up conditions.

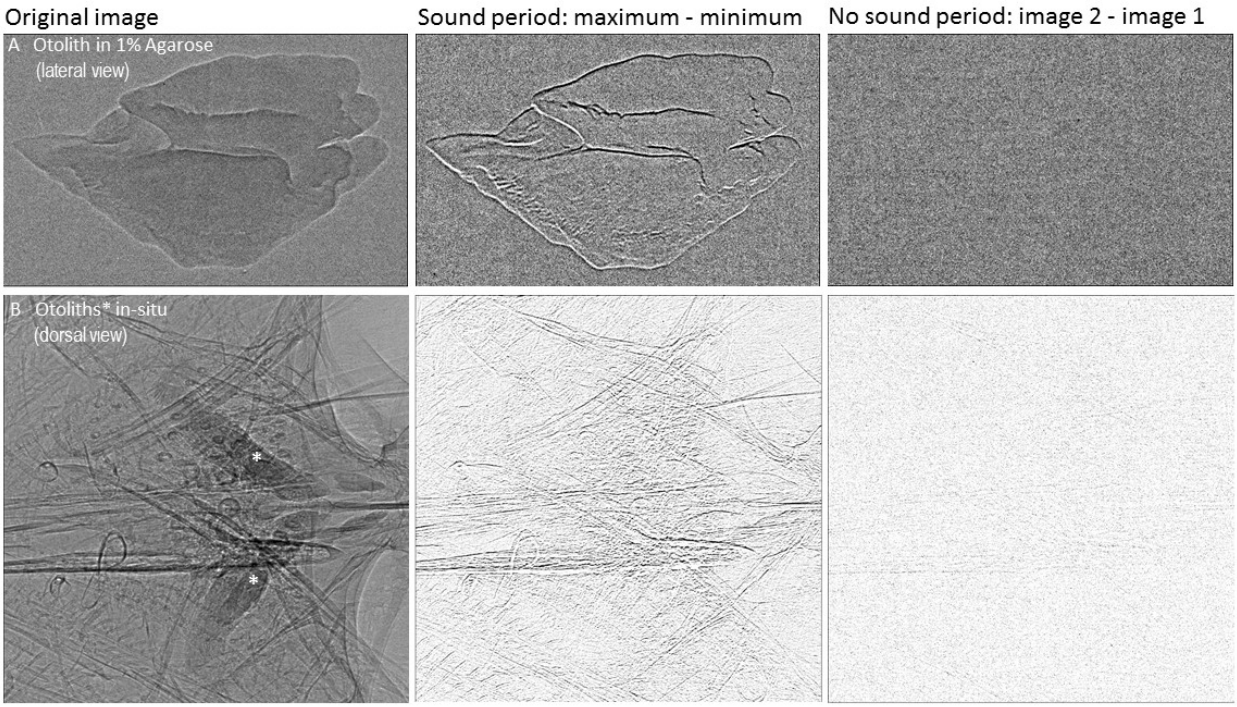


**Fig. 2.** The single *E. maculatus* sagitta (A) as well as otoliths in the whole fresh-dead specimen of *E. maculatus* (B) show a distinct motion during the sound period (middle column) whereas in the no sound period structures do not show any clear movement (right column).

Conclusions & Outlook:

The measurements at ID19 provided high quality data that allow characterizing for the first time otolith motion in whole fishes using a non-invasive imaging technique. At the moment, we are writing up a manuscript entitled "First visualization of the in-situ motion of otoliths in the fish ear" which we will submit to PLoS Biology or Current Biology. Further experiments could test the effects (1) of 0.1 vs. 0.2 kHz stimuli and (2) of decreasing sound pressure levels during the stimulus presentation (down to 120 dB re 1 µPa).