


The development of hearing abilities in the shark *Scyliorhinus canicula*

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Abstract

The few works on audition in sharks and rays concern only adult specimens. We report the hearing abilities in the dogfish *Scyliorhinus canicula* at different stages, from embryos that still have their yolk sac inside their egg, to juveniles. Hearing development corresponds to an increase in the frequency range from 100–300 Hz in early pre-hatching stages to 100–600 Hz in juveniles. Modifications in hearing abilities correspond to the development of the brain, the increase of the volume of the membranous labyrinth, the growth of the sensory epithelium, and the development of stereocilia in addition to kinocilium before hatching. This work offers solid insights into the development of hearing abilities that usually can only be inferred from the anatomy of vertebrates or after birth/hatching. It shows also that shark can be sensitive to background noise during development.

KEYWORDS

audition, dogfish, labyrinth, macula, ontogeny

1 | INTRODUCTION

Hearing abilities in sharks, skates and rays have been measured for approximately 10 elasmobranch species, in contrast to more than 100 bony fish species (Ladich and Fay, 2013). All tested elasmobranchs cannot detect sounds above 1 kHz (Ladich and Fay, 2013).

The morphology and functional anatomy of the inner ear of elasmobranchs have already been described in different species (Löwenstein and Sand, 1940; Lowenstein and Roberts, 1951; Tester *et al.*, 1972; Barber and Emerson, 1980; Corwin, 1981a; Maisey, 2001; Lovell *et al.*, 2007). Each inner ear can be divided into two parts forming a membranous labyrinth. The upper part consists of three semicircular canals (Barber and Emerson, 1980; Maisey, 2001; Lovell *et al.*, 2007; Mills *et al.*, 2011) that are each associated with an ampulla housing both supporting cells and hair cells grouped in a sensory epithelium called crista (Montgomery *et al.*, 2006; Lovell *et al.*, 2007). In addition, elasmobranchs possess a fourth canal (the endolymphatic duct) that connects the membranous labyrinth of the inner ear to the external environment (Tester *et al.*, 1972; Mills *et al.*,

2011). The lower part of the inner ear is made up of three chambers (i.e. the saccule, lagena, utricle) that contain thousands of sensory hair cells forming the sensory macula (Lowenstein and Roberts, 1951; Corwin, 1981a; Maisey, 2001). These maculae are overlain by calcium carbonate granules or otoconia (Tester *et al.*, 1972; Corwin, 1989) that act as an inertial mass to stimulate the hair cells (Tester *et al.*, 1972; Hueter *et al.*, 2004). Besides this otolithic-pathway, elasmobranchs possess an additional end organ, the sensory macula neglecta, located in the posterior canal duct in the dorsal portion of the saccule (Retzius, 1884; Corwin, 1981a).

Oviparous females of the lesser-spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758) lay quadrangular horny eggshells provided with tendrils that allow their attachment to weed or stones. Eggs incubate for about 6–8 months. The development, from fertilisation to hatching, can be divided into 34 stages (Ballard *et al.*, 1993). At the beginning of the development, the oxygen needs of the embryo are satiated thanks to the permeability of the eggshell to water (Kormanik, 1993). At about 3–4 months (about 40 mm long), the embryo becomes more active in its eggshell and simple oxygen

diffusion through the shell is not sufficient. Pre-hatching then consists in the early opening of the eggshell (Demski *et al.*, 1973). This allows the flow of water into the shell to increase the uptake of oxygen (Diez and Davenport, 1987). At the pre-hatching stage, it is experimentally possible to culture the embryo and its yolk sac in sea water outside the eggshell (Fouley and Mellinger, 1980). Definitive hatching finally occurs about 3 or 4 months after pre-hatching, at a size of 90–100 mm (Ballard *et al.*, 1993; Mellinger, 1994).

This pre-hatching phenomenon provides an exceptional opportunity to follow the early development of the inner ear without damaging the fish. We investigated the physiological aspects of the auditory system in the small-spotted dogfish *S. canicula* using different developmental stages (i.e. from early embryos to juveniles), and we also studied the morphological development in the same specimens.

2 | MATERIALS AND METHODS

All procedures and methods were approved by the ethical commission of the University of Liège (ethics case 1677).

2.1 | Acquisition and maintenance of sharks

The different developmental stages (i.e. 31, 33 and 34) were identified according to the study by Ballard *et al.* (1993). A total of 26 individuals (total length, TL: 50–465 mm) were used in this study. Twenty-one pre-hatching embryos, from stage 31 (50–62 mm TL), stage 33 (65–90 mm TL) and stage 34 (90–105 mm TL), were obtained from the Sea Life Centre and the Roscoff Marine Station. In addition, five juveniles (340–465 mm TL) were obtained from the Aquarium Dubuisson. Eggs and juveniles were maintained at the ULiège facilities (Belgium) in tanks (0.6 × 0.7 × 1.5 m) filled with saltwater maintained at 16.9°C. These tanks were equipped with a sand bottom and external filters. No internal filters or air stones were used in order to create a quiet acoustic environment. Sharks were kept under a 12:12 hr L:D photoperiod, and juveniles were fed with squid every day. All individuals were held for 1–3 days before being tested.

2.2 | Auditory-evoked potential threshold measurement: experimental set-up

The auditory-evoked potential (AEP) technique directly measures nerve impulses created in the eighth nerve, and activity of the brainstem evoked by acoustic stimuli (Bullock and Corwin, 1979; Corwin and Northcutt, 1982). The presence or absence of a response to sounds of different intensities and frequencies allows the measurement of AEP thresholds. The experimental set-up was similar to that used for previous studies (Parmentier *et al.*, 2009; Colley *et al.*, 2013; Kéver *et al.*, 2014). No anaesthetics or neuromuscular-blocking drugs were used during the AEP recordings. However, all individuals

(embryos and juveniles) were restrained in a custom-made device in order to prevent electrodes dislodging, limiting body and tail movements while allowing normal ventilation. Note that embryos were removed from their eggshell for the experiment. Three subdermal stainless-steel needle electrodes (Rochester Electro-Medical) were used for recording the AEP signal. These electrodes were coated with nail polish so that only ~1 mm of metal was exposed at the tip. The recording electrode was inserted about 1–2 mm into the head over the otic region, the reference electrode was inserted into the epaxial musculature, and the ground electrode was placed in the tank water near the shark. All AEP recordings were carried out in a steel barrel (0.78 m high, 0.48 m diameter, 3 mm thickness) oriented vertically, closed at the bottom and opened at the upper part. The steel barrel was filled with saltwater (T = 16.9°C) up to a height of 0.73 m, and the test specimen was suspended 20 cm below the water surface with the head placed at the centre of the circle. So, the head shark was about 0.5 m above the loudspeaker (UW-30, Lubell Labs) placed on a rubber support on the bottom of the experimental tank. The entire set-up was enclosed in a walk-in soundproof booth (interior dimensions: 1.8 × 1.8 × 2.1 m).

2.3 | Stimulus generation and AEP recordings

All hearing is based on mechanosensory hair cells transducing vibrations (and not pressure) into electrical signals (Nedelec *et al.*, 2016). It has already been demonstrated that fish species without swim bladders also lack sound pressure sensitivity (Popper and Fay, 2011; Ladich and Fay, 2013). However, sound pressure is always associated with particle motion (Gans, 1992), meaning the sonic stimuli can be used to highlight the ability to detect different frequencies.

The presentation of sound stimuli and the determination of thresholds followed the detailed description given by Parmentier *et al.* (2009). Stimuli were tone bursts of 50 ms in total duration, gated with a Hanning window. The phase of the tone was alternated between presentations to minimise electrical artefacts from the recordings. Test frequencies ranged from 100 to 1500 Hz. During each trial, 11 different frequencies were presented: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 and 1500 Hz; these frequencies covered the expected range of hearing of the studied species (Ladich and Fay, 2013). Sounds were attenuated in 6-dB steps beginning at the loudest level that could be generated at each frequency. Evoked potentials recorded by the electrode were fed through a TDT HS4-DB4 amplifier (10 000 gain) connected to an RP2.1, routed into the computer and averaged using BioSig software. To measure the evoked response at each level of each frequency, the signal was presented 500 times. Sound levels produced by the loudspeaker were calibrated with a Brüel and Kjær (Nærum, Denmark) hydrophone (model #8101; sensitivity -184 dB re 1 V/1 µPa; frequency response 0.1–200 kHz) placed in the experimental tank at the position normally occupied by the shark's head. The hydrophone was connected to a calibrated Brüel and Kjær 2610 amplifier that gave the absolute sound pressure level (SPL) at all frequencies and intensity levels tested.

A 4096-point Fast Fourier Transform (FFT) was used to analyse the averaged AEP waveforms in the frequency domain. An auditory response was visually determined to be positive if the signal showed the presence of a peak at twice the stimulus frequency (e.g. 400 Hz peak when the signal played was 200 Hz), which is typical of fish AEP (Mann *et al.*, 2001; Parmentier *et al.*, 2009). The background level was estimated from the AEP power spectrum with a window of 100 Hz around the doubling frequency (Casper and Mann, 2006). Thresholds were determined by both the averaged AEP trace and power spectrum, and were defined as the lowest sound level to show a repeatable AEP trace above the background noise, with an FFT peak at twice the stimulus frequency being at least 3 dB above the background level (Egner and Mann, 2005).

To ensure that the recorded AEP traces were not artefacts, controls were run by testing dead individuals in the experimental set-up. These dead sharks were embryonic specimens that were killed. No responses were recorded with dead sharks.

2.4 | Morphology of the inner ear

One individual of each of the pre-hatchling stages (31, 33 and 34) was fixed in 5% formaldehyde for X-ray microtomography. A three-dimensional study of the inner ear morphology and the brain was performed at the Centre for Microtomography of the University of Poitiers (France) in order to make a comparison between these different developmental stages. Specimens were treated with phosphomolybdic acid-dye stain 2.5% (PMA) for at least 5 days in a volume corresponding to 10% of the head volume. In addition, one juvenile that had been preserved in ethanol was rinsed and rehydrated in phosphate-buffered saline (PBS) for 24 h before being fixed in 5% formaldehyde for 24 h, and then treated with PMA. X-ray microtomography of all specimens was performed according to the protocols reported by Boistel *et al.* (2011) and Zanette *et al.* (2014) using an RXsolutions (Annecy, France) EasyTom XL Duo microtomograph. The reconstruction was performed using the software XAct (RX solution) with the FDK algorithms. Three-dimensional (3D) images were produced in 16-bit and subsequently converted into 8-bit voxels using ImageJ (Abramoff *et al.*, 2014). Three-dimensional processing and rendering according to the protocols reported by Zanette *et al.* (2014) were obtained after semi-automatic segmentation of the body, brain and inner ear using 'generated surface'. Direct volume renderings (iso-surface reconstruction) were used to visualise the subset of selected voxels of body, brain and inner ear in AVIZO 8.0.1 (FEI, VSG, Merignac, France, <http://www.fei.com/software/avizo3d/>), after having used Material Statistics to obtain the volume measurements for all segmented components.

2.5 | Histological study of the inner ear

Three individuals of each of the pre-hatchling stages (31, 33 and 34) were used to compare the general morphology of their auditory

apparatus as well as the development of the sensory hair cells of their saccular macula. First, one specimen of each stage was dehydrated in butanol, decalcified, embedded in paraffin and serially sectioned using a Reichert microtome (7 μ m thickness). Each section was stained with Masson's Trichrome. The general morphology of the auditory apparatus was examined with a Wild M10 (Leica) binocular microscope. Sections were observed using an Olympus polarising microscope (Leica DM 100). Then, the otic capsules were removed from all the other individuals and fixed in 2–5 volumes of fixative solution containing 5% glutaraldehyde in a buffer composed of 0.2 M sodium cacodylate, 0.25 M sodium chloride, 10 mM calcium chloride and 1% sucrose at pH 7.4–7.6 (Corwin, 1983). The otic capsules were stored for up to 1 month in refrigerated fixative. The saccular macula was then dissected from each of the otic capsules and post-fixed in 1% osmium tetroxide in the above buffer. These were dehydrated in an ethanol series, dried by the critical-point method with carbon dioxide using a Leica EM CPD300 critical point dryer, and examined with an ESEM Quanta 600 (FEI™) scanning electron microscope (SEM) at 20.0 kV. Hair cells of the saccular macula were photographed using the SEM in order to make a comparison of their developmental stage.

No juveniles were used in this experiment because we were authorised to kill only one specimen out of five juveniles; other specimens had to be kept alive and returned to the Aquarium Dubuisson (Liège, Belgium). The only available specimen was used for the X-ray microtomography.

2.6 | Statistical analyses

The Bartlett's test was used to test the homogeneity of variances between groups of different developmental stages. As the assumption of homogeneity of variances was not always met, a non-parametric Kruskal–Wallis test was applied to compare auditory thresholds expressed in terms of SPLs between the different developmental stages. Eventually, non-parametric Mann–Whitney *U*-tests with Bonferroni adjustments were used to make pairwise comparisons of frequencies for which the significance level was met by the Kruskal–Wallis test. All statistical analyses were carried out with RStudio. Results are presented as means \pm SD. In addition to probabilities (*P*-values), effect sizes of each statistical measure were estimated as the difference between medians of multiple groups using the web application Estimation Stats (<http://www.estimationstats.com>; Ho *et al.*, 2019) before inferences and conclusions were drawn.

3 | RESULTS

In agreement with previous statements, we do not claim the sharks were able to detect sound pressure. We obtained evoked potentials from all developmental stages tested; we simply report they were all exposed to the same sound pressure but show different responses,

most probably because the particle acceleration related to each corresponding sound was different. It is, however, not possible to infer precisely the values of particle motion in the framework of this study (Rogers *et al.*, 2016). At this stage of the knowledge, it is important to note we had a signal at the time of sound invoice for each of the 26 tested specimens, whereas we did not obtain any response from dead specimens. Moreover, specimen response was different according to the used frequency. These responses were coherent within specimens being at the same stage.

Audiograms showed a gradual increase in the frequency range with increasing age (Fig. 1): the detectable frequencies ranged from 100 to 300 Hz in stage 31 (pre-hatching stage), from 100 to 400 Hz in stages 33 and 34 (hatching stage), and from 100 to 600 Hz in juveniles. No response was detectable at 700, 800, 900, 1000 and 1500 Hz for any stage of development.

In the audiograms expressed in SPLs, all developmental stages (except stage 31) showed their best hearing sensitivity at the highest detectable frequency (Fig. 1a).

Hearing thresholds at 200 Hz were significantly different (Kruskal–Wallis test, $\chi^2_4 = 18.15$, $P = 0.001$) between stages 31 and 33 (Mann–Whitney U -test, $P = 0.027$), and between stages 31 and 34 (Mann–Whitney U -test, $P = 0.02$). At 300 Hz (Kruskal–Wallis test, $\chi^2_4 = 13.37$, $P = 0.009$) and 400 Hz (Kruskal–Wallis test, $\chi^2_4 = 10.96$, $P = 0.012$), only stages 33 and 34 differed significantly (300 Hz: Mann–Whitney U -test, $P = 0.048$; 400 Hz: Mann–Whitney U -test, $P = 0.046$). No other significant difference was found at any tested frequency between the different stages (Kruskal–Wallis test, $\chi^2_4 = 0.54$ – 7.7 , $P = 0.052$ – $.46$). Effect size calculation confirmed that stages show little differences in their auditory thresholds when comparing them for a given frequency. The effect sizes were small as they ranged from 0 to 7 dB re:1 μ Pa. Larger values were only found at 200 Hz between stages 31 and 34 (11 dB re: 1 μ Pa), and at 300 Hz between stages 33 and 34 (9 dB re: 1 μ Pa; Table 1).

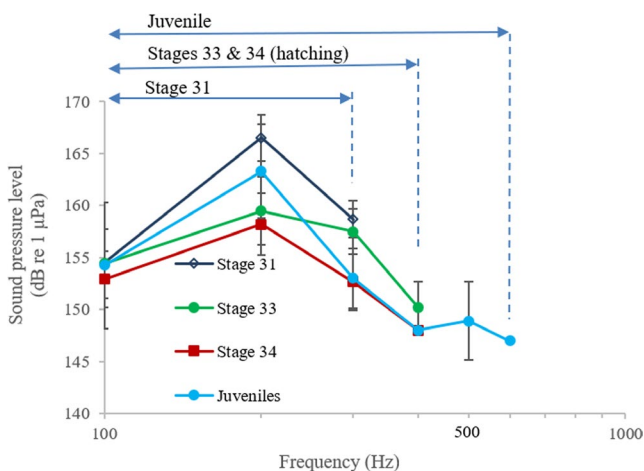


FIGURE 1 Hearing threshold mean (\pm SD) of the different studied stages in *Scylliorhinus canicula*. Responses expressed in terms of sound pressure level (SPL). Double arrows help to visualise the increase in the frequency range

In the next step, we followed the macroscopic development of the brain and inner ear. At the pre-hatching stage, all the structures classically found in the inner ear (otolithic chambers, the three semi-circular canals and their ampullae) are already present. Only the sizes of these inner ear structures seem to be different between the developmental stages (Fig. 2). The five regions commonly found in the brain of elasmobranchs are also already visible at the pre-hatching stage (Fig. 2).

All these data support the fact that we were able to detect hearing abilities, but histological examinations were required to refine the results. The different sensory epithelia (i.e. crista in the ampullae, maculae in the otolithic chambers, and macula neglecta in the posterior canal duct) are already present (Figs 3 and 4), and clearly increase in size between pre-hatching and hatching stages. Although this comparison needs to be carefully addressed, it seems that the number of sensory hair cells also increases with age. These hair cells also become more elongated, uniform and their nucleus is centred (Fig. 3). Moreover, a layer of non-sensory supporting cells appears to be more structured at hatching than at pre-hatching stage (Fig. 3).

Interestingly, the increase of auditory capabilities with age is also supported by the development of the saccular macula sensory hair cells observed using the SEM (Fig. 5). Hair cells are mainly composed of a single kinocilium and only a few short stereocilia at pre-hatching stage (Fig. 5). At the hatching stage, the saccular macula contains numerous hair cells with well-developed stereocilia (Fig. 5). We were not able to make a quantitative comparison of sensory hair cells between the different developmental stages.

4 | DISCUSSION

In *Carcharhinus melanopterus* and *Raja clavata*, the size of the macula neglecta and the number of hair cells grow with age (Corwin, 1981b, 1983). About 80% of the number of macula neglecta hair cells in the adults of *C. melanopterus* are produced post-embryonically (Corwin, 1981b). In this study, the youngest organisms (stage 31, 3–4 months before hatching) already possess the three otolithic chambers, the three semi-circular canals as well as their ampullae, namely all features allowing them to detect sounds from 100 to 300 Hz. Juveniles are able to detect sounds from 100 to 600 Hz. Only the size of these structures increases with age. Our morphological study based on both 3D reconstructions of the inner ear and histological sections supports the hypothesis that the development of the frequency range takes place simultaneously with the growth of the inner ear (Figs 3 and S1) and the brain (Fig. 2). This improvement of inner ear sensitivity takes place not only inside the egg but also after hatching, as juveniles possess better hearing abilities than embryos. However, the development of other anatomical elements such as nerves, parietal fossa (Bullock and Corwin, 1979), statoconia (Hanson *et al.*, 1990; Lychakov *et al.*, 2000) or lateral line (Higgs and Radford, 2013) can be involved in the detection of particle motion, and all deserve additional studies.

However, the development only appears to be related to the frequency range. Older specimens do not have improved sensitivity

TABLE 1 Summary of effect size calculated as the difference between medians and exact *P*-values of all pairwise comparisons made using Mann–Whitney *U*-tests comparing SPL values between different developmental stages for all tested frequencies

Group 1	Group 2	Group 1 N	Group 2 N	Difference	ci_lower_limit	ci_upper_limit	<i>P</i> -value
100 Hz							
Stage 31	Stage 33	7	7	0	-5.5	-5.5	1
Stage 34	Juveniles	7	5	0	-5.5	0	1
Stage 31	Stage 34	7	7	0	0	0	1
Stage 33	Juveniles	7	5	0	-10.5	0	1
Stage 31	Juveniles	7	5	0	-5.5	0	1
Stage 33	Stage 34	7	7	0	-10.5	0	1
200 Hz							
Stage 31	Stage 33	7	7	-5	-5	-5	0.027
Stage 34	Juveniles	7	5	6	6	6	0.921
Stage 31	Stage 34	7	7	-11	-11	-11	0.02
Stage 33	Juveniles	7	5	0	0	0	1
Stage 31	Juveniles	7	5	-5	-5	-5	0.067
Stage 33	Stage 34	7	7	-6	-6	-6	0.373
300 Hz							
Stage 31	Stage 33	3	7	4	-4	4	1
Stage 34	Juveniles	7	5	5	-5	5	1
Stage 31	Stage 34	3	7	-5	-14	-5	0.844
Stage 33	Juveniles	7	5	-4	-9	-4	0.14
Stage 31	Juveniles	3	5	0	-9	0	1
Stage 33	Stage 34	7	7	-9	-14	-14	0.048
400 Hz							
Stage 34	Juveniles	7	5	0	0	0	1
Stage 33	Juveniles	7	5	-4.5	-4.5	-4.5	0.113
Stage 33	Stage 34	7	7	-4.5	-4.5	-4.5	0.046
Adults	Stage 33	2	7	4.5	4.5	4.5	0.0769
500 Hz							
Stage 34	Juveniles	3	5	0	0	0	1
Stage 33	Juveniles	2	5	0	0	0	1
Stage 33	Stage 34	2	3				

Missing values result from low sample size and/or low variability within a group. *N* corresponds to the number of specimens. Bold data refers to results that were significantly different.

and do not have a better threshold for a given frequency. Changes observed in the maculae and the larger frequency range in older *S. canicula* are consistent with observations on *C. melanopterus* and *R. clavata* (Corwin, 1981b, 1983). The development of a broader spectrum of detected frequencies during development in the egg was also found in the clownfish *Amphiprion ephippium* and *Amphiprion rubrocinctus* (Simpson *et al.*, 2005).

The shark is sensitive only to particle motions associated with a sound field, and not sound pressure. Studies dealing with particle motions are scarce mainly because we lack appropriate equipment and expertise. Methods to measure and model the particle motion are still needed to understand better the acoustic communication, and the potential effects of noise on aquatic fauna (Nedelec *et al.*,

2016; Rogers *et al.*, 2016). We provide hearing data in relation to near field sound pressure. We are aware this method has the potential to introduce experimental error because it does not perfectly mirror particle motions (Nedelec *et al.*, 2016; Rogers *et al.*, 2016), and deviations between sound pressure and particle motion can be high in the near field, i.e. near sound sources (Nedelec *et al.*, 2016). However, this error would be minimal because we always use the same species in the same experimental approach and in the same acoustic environment (Ladich and Fay, 2013; Rogers *et al.*, 2016; Popper *et al.*, 2019). Size could be a problem in testing the hearing abilities in fish (Rogers *et al.*, 2016), but sharks do not have swim bladders and consequently do not introduce acoustic scatterer in the tank, limiting variation in the near sound field.

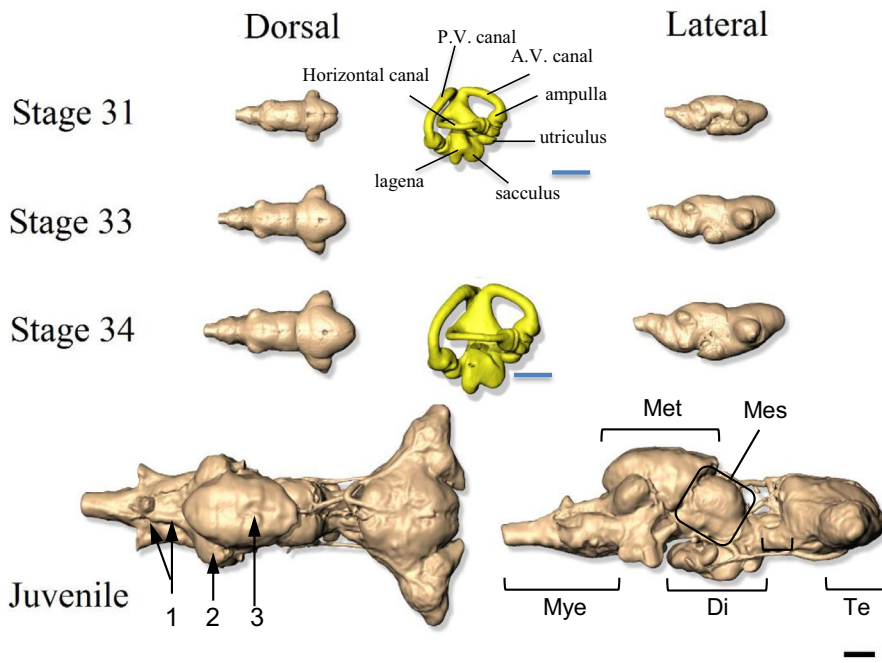


FIGURE 2 3D reconstruction of the brain in *Scyliorhinus canicula* from pre-hatching stages (31, 33 and 34) to juvenile stage obtained by X-ray microtomography. 1: Lateral line and electroreceptive lobes; 2: auricle of cerebellum; 3: cerebellum; A.V., antero-ventral; Di, diencephalon; Mes, mesencephalon; Met, metencephalon; Mye, myelencephalon; P.V., postero-ventral; Te, telencephalon. Scale bar: 2 mm

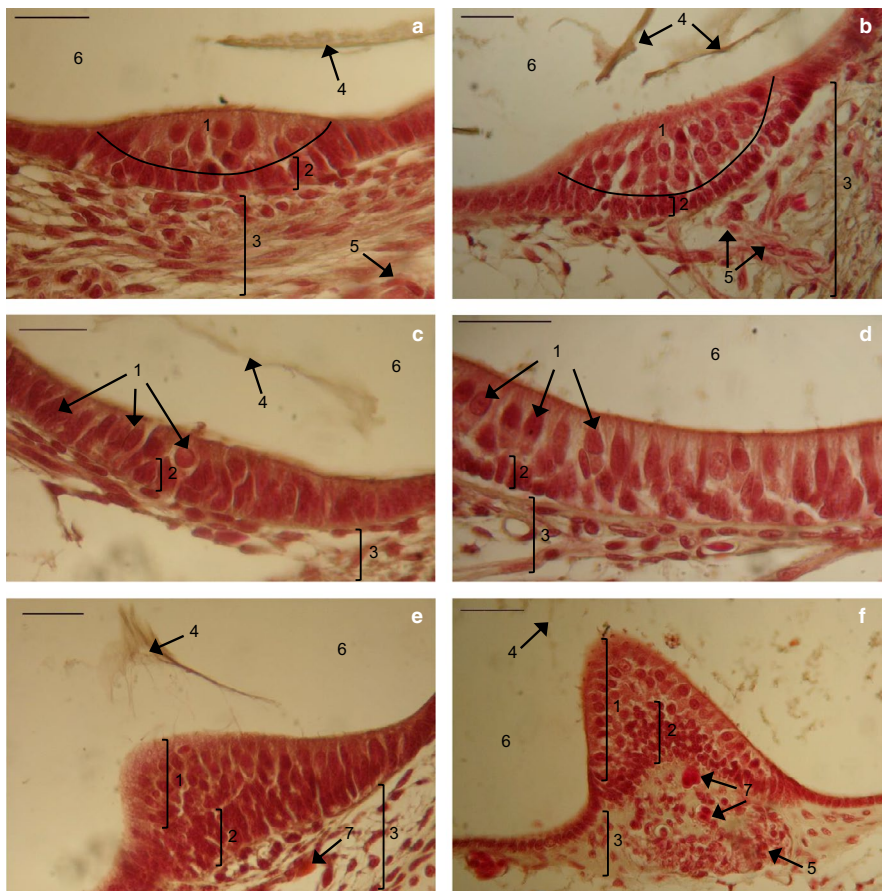


FIGURE 3 Comparisons between stages 31 (left) and 34 (right) of the maculae in the sacculus (a, b), in the lagena (c, d), and of the crista in ampullae of semi-circular canals (e, f). 1: Hair cells; 2: supporting cells; 3: stalk of the crista (connective tissue); 4: cupula; 5: nerve terminals; 6: lumen; 7: blood vessel. Scale bars: 30 μ m

At this stage, we are not fully interested in knowing what the real threshold is but how it evolves in parallel with inner ear development. Many studies have shown there is great variability in sound level detection within the same species determined by different

investigators (Popper *et al.*, 2019), but it is not the case for the frequency range. Five–seven specimens per developmental stage were investigated. Within each stage, these specimens provided the same kind of response, i.e. they were able to detect some frequencies or

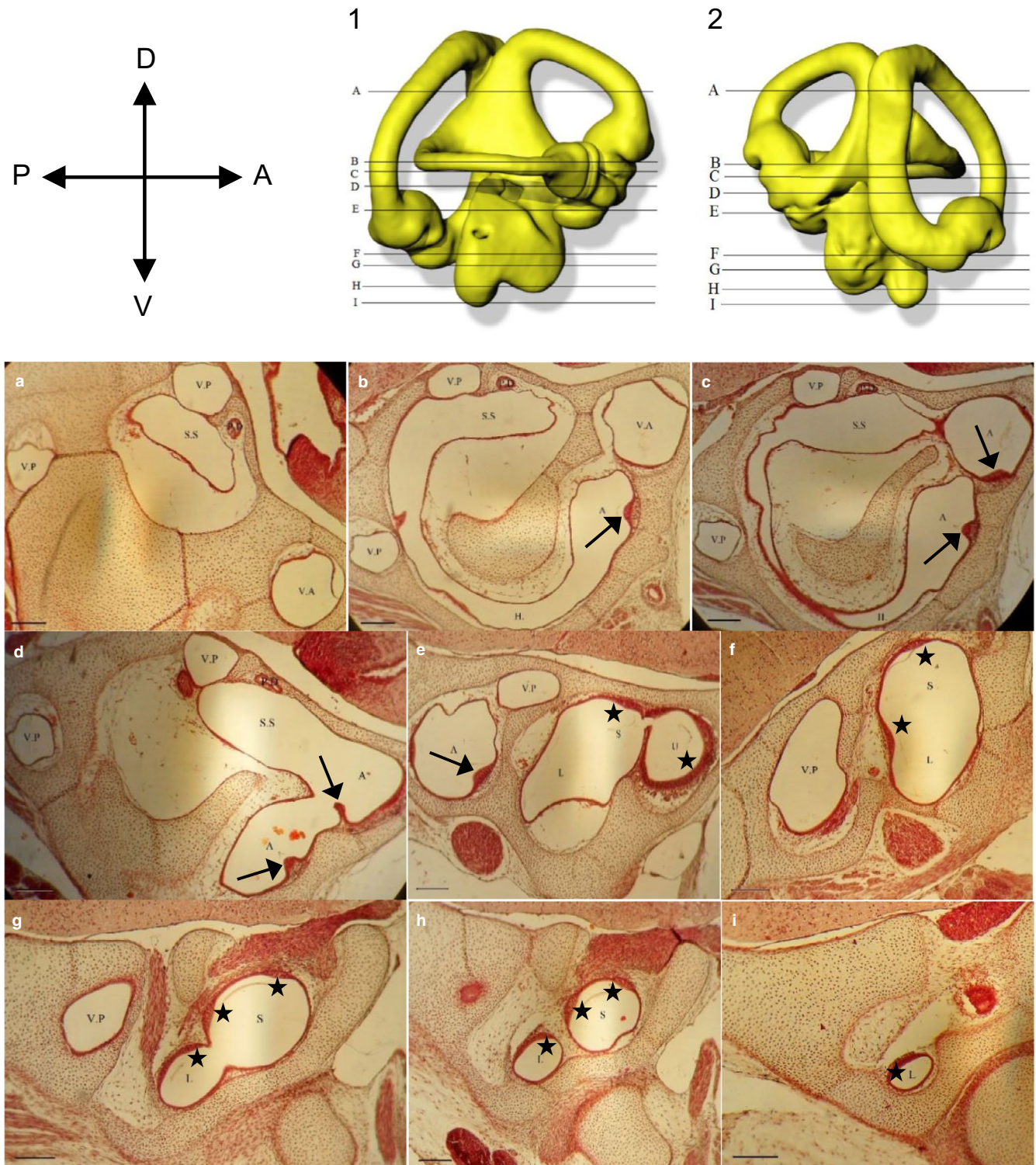


FIGURE 4 Frontal histological sections of the right inner ear in *Scyliorhinus canicula* at stage 31. Letters refer to the section indicated on the 3D reconstruction (1: lateral view; and 2: medial view) of the inner ear obtained by X-ray microtomography. Each anatomical feature is labelled as follows: A, ampulla; L, lagena; H., horizontal semi-circular canal; P.D., posterior canal duct; S, sacculus; S.S., sinus superior; U, utriculus; V.A., vertical anterior semi-circular canal; V.P., vertical posterior semi-circular canal. Arrows in b, c, d, e indicate cristae in the ampullae of semi-circular canals. Stars in e, f, g, h, i indicate the saccular, lagenar or utricular maculae. Orientation is given by the notations: A, anterior; D, dorsal; P, posterior; V, ventral. Scale bars: 150 μ m

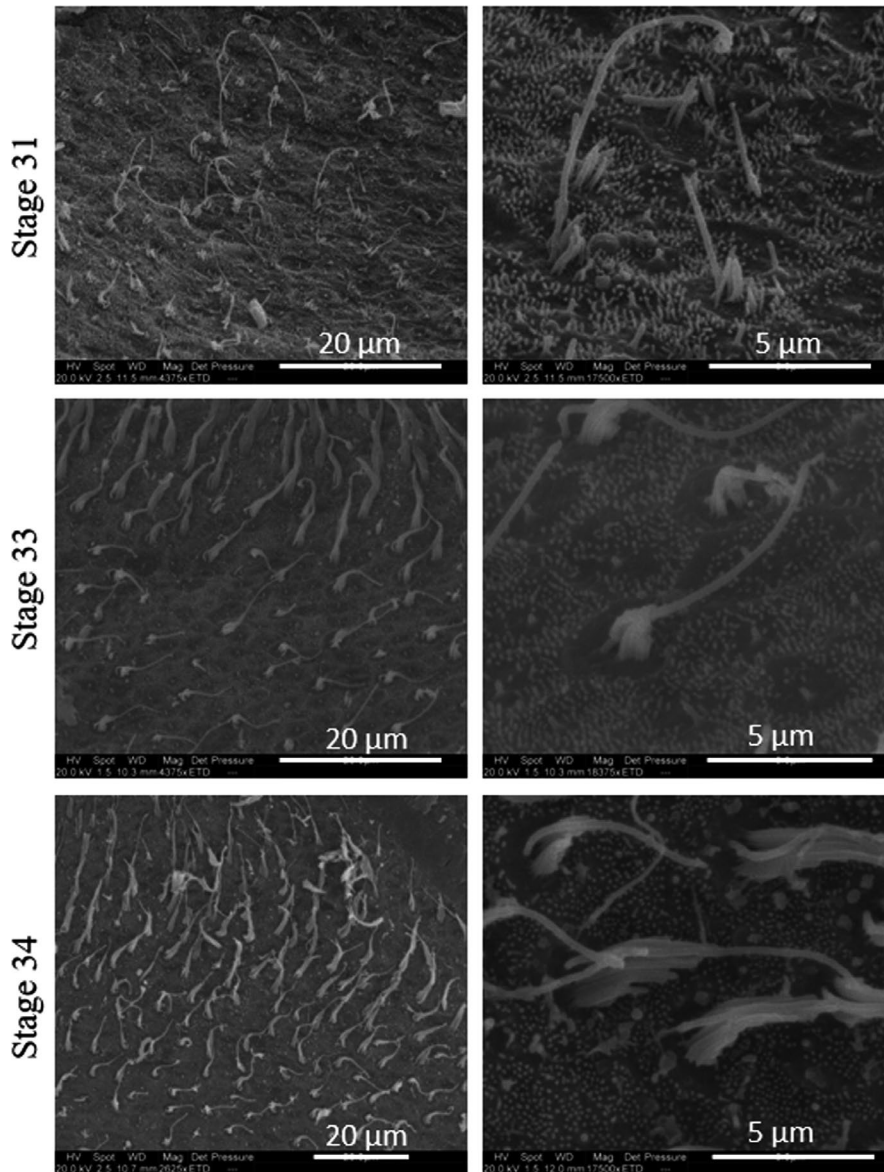


FIGURE 5 Scanning electron micrograph (SEM) of macula sensory hair cells in *Scyliorhinus canicula* at stages 31 (3–4 months before hatching), 33 and stage 34 (hatching). Stage 31 shows some hair cells with their well-developed kinocilium and short stereocilia. Stage 34 shows hair cells with well-developed stereocilia

not. Our results highlight the ear development in sharks corresponds to an enlargement of the frequency bandwidth.

5 | CONCLUSION

This study shows that the shark *S. canicula* is able to detect sounds within its eggshell, and the development of hearing capacities takes place simultaneously with the development of sensory epithelia. It has the main advantage of highlighting that physiological experiments on auditory abilities can be done before hatching in these oviparous species. It should provide new insight on the study of hearing development.

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CONFLICTS OF INTEREST

The authors do not have any conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

EP conceived and designed the experiments. OC and MB performed the hearing experiments. RB performed the CT scan and 3D constructions. OC, MB, PC performed histological study. FB performed statistical analysis. EP and OC wrote the manuscript; other authors provided editorial advice.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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