

# Otoconial Mass of the Bullfrog Sacculus

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In order to study the effects of mechanical loads on hair-bundle function, one must consider the effects of accessory structures in the intact organ. In the sacculus, hair bundles protruding from the sensory epithelium are coupled to an overlying otolithic membrane. Sitting atop this membrane is an otoconial mass, divided in two along its striola. While we have estimates of the elastic load imposed by the otolithic membrane ( $1350 \mu N \cdot m^{-1}$  [1]), the mass load imposed by the overlying otoconia has not been reported.

Here we measure the otoconial mass of the sacculus of the American bullfrog, *Rana catesbeiana*. Measurements were taken for two ears in two female frogs. Prior to dissection, we measured the mass of a small petri dish in three independent measurements, yielding  $1.74430 \pm 0.00002$  g. Each sacculus was dissected and excess tissue removed, leaving only otolith in the petri dish after completion of this process. We let the contents dry, covered, over a period of five hours. We next measured the mass of the petri dish with added otoconia in three independent measurements, yielding  $1.75510 \pm 0.00008$  g. This yielded a difference of  $0.0108 \pm 0.0001$  g.

Given these data, we find a mass of approximately 0.0054 g for the otoconial mass of a single sacculus. Calcium carbonate, the primary component of this mass, has a density of  $2.71 \text{ g} \cdot \text{cm}^3$ . With a mass of 5.4 mg, this yields a sphere with a radius of 781  $\mu m$ . This value is within the range of the sacculus of the bullfrog, which has a diameter of approximately 600-900  $\mu m$ .

The otoconial mass is suspended in endolymphatic fluid. Thus, the apparent mass of the otolith is smaller due to buoyant forces. If we take the density of the surrounding fluid to be equal to the density of water ( $1000 \text{ kg} \cdot \text{m}^3$ ), the apparent mass of the otolith becomes:

$$\begin{aligned} m_{oto} - m_{oto,apparent} &= \rho_{H_2O} \times V_{oto} , \\ 5.4 \times 10^{-6} \text{ kg} - m_{oto,apparent} &= 1000 \text{ kg} \cdot \text{m}^3 \times \frac{4}{3} \pi (781 \times 10^{-6} \text{ m})^3 , \\ m_{oto,apparent} &= 3.4 \text{ mg} , \end{aligned}$$

which is 63% of the unsuspended mass of the otolith. This value can also be found from the ratio of the densities of water and calcium carbonate ( $1 - \frac{1}{2.71} \text{ g} \cdot \text{cm}^3 = 0.63$ ).

The adult bullfrog sacculus is estimated to have roughly 3,000 hair cells [2]. For younger frogs, the span of the sacculus is roughly half that of the adult. If we assume a total of roughly 1,500 hair cells, one might expect an individual hair bundle to feel the effect of approximately 3.6  $\mu g$  of mass load if the load were equally distributed and unsuspended or an apparent mass of 2.3  $\mu g$  when suspended in water. If we assume a stiffness of 1000 to 1350  $\mu N \cdot m^{-1}$  and use the apparent mass of the otolith, this yields characteristic frequencies of:

$$f = \frac{1}{2\pi} \sqrt{\frac{1000 \times 10^{-6} \text{ N} \cdot \text{m}^{-1}}{2.3 \times 10^{-9} \text{ kg}}} = 105 \text{ Hz and}$$

$$f = \frac{1}{2\pi} \sqrt{\frac{1350 \times 10^{-6} N \cdot m^{-1}}{2.3 \times 10^{-9} kg}} = 122 Hz ,$$

values within the range of best frequencies measured from saccular afferents in vivo [3]. If the mass of the otolith out of suspension were used in the above equations, this would yield a frequency range of 84-97 Hz, also within the expected frequency range. Given a frequency range of 35-90 Hz (Figures 2A-5A from [3]) and a stiffness of  $(1000 \mu N \cdot m^{-1})$ , this yields an estimated mass range of 3-21  $\mu g$  per hair bundle. Assuming the stiffness value reported here is accurate, the mass value reported here may be an underestimate of the total mass of the otolith in vivo. Alternatively, the mass distribution may be unequal. This could have the effect of increasing the mass load on an individual bundle, thus decreasing its resonant frequency.

This mass measurement is contrasted with the 35-100  $ng \cdot row^{-1}$  experienced by outer hair cells coupled to the tectorial membrane within the cochlea of the Mongolian gerbil [4]. Perhaps one might expect the mass load between an auditory and vestibular organ to differ by 40-100 times, and this result may thus be unsurprising. Nonetheless, it is important to note that a study of the effects of mass loading a vestibular hair bundle may require values much greater than those experienced by hair bundles of auditory and lateral-line organs.

## References

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4. Edge et al. (1998) Morphology of the unfixed cochlea. *Hearing Research* 124(1-2):1-16.